I. REMARKS

A. Status of the Claims

Claims 1-7, 12, 14, 20, 23-25, 27, 30, 32, 63-65, and 67-70 are pending and under examination. No claims are amended or canceled.

B. Interview Summary

Applicant's representative thanks Examiner Leith for conducting a telephone interview on December 3, 2009.

Applicant's representative indicated to the Examiner that the Applicant had noted that the Examiner had cited publications related to "pawpaw" Asimina species. However, Applicant has noted that the term "pawpaw" or "paw paw" is used in Australia and other locations in the British English-speaking world to clearly and definitely refer only to "pawpaw" Carica species. Further, Applicant notes that the terms "pawpaw" and "papaya" are both used to refer only to Carica species in Australia and elsewhere in the British English-speaking world.

Applicant also notes that generically claimed subject matter in the application is not limited to a particular species of fruit and/or vegetable. Also, all uses of the term "paw paw" in the specification were intended to clearly and definitely refer only to the "pawpaw" Carica species.

Optional strategies for either amending the specification or submitting arguments or

evidence on the record regarding use of the term "pawpaw" were also discussed with the Examiner. The Examiner indicated that any amendments regarding the term "pawpaw" would be considered and the issue of new matter added by amendment would be determined at that time.

Applicant provides additional information regarding the use of the term "papaw" at the end of this response and in the attached expert's declaration.

C. Enablement Rejections Under 35 USC 112, 1st paragraph

Claims 12, 14, 65, and 67 are rejected under 35 U.S.C. § 112, first paragraph, as non-enabling because the claims "contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." Office Action, page 3. Applicant respectfully traverses this rejection for reasons of record, and as supplemented below

The Office alleges "It has not been demonstrated, in the Original disclosure, where an extract or fruit of pawpaw alone alleviated any wound as required by claims 12-14." *Id.*, page 4. In particular the Official Action states at page 5:

> The only examples in the specification which use the product of Example (1) alone are Example 18 (topical use for pain), Example 19(a) (topically for dry skin as a moisturizer), Example 19(b) (topical pain treatment) and Example 21 (topical, moisturizing). The state of the art is unpredictable with regard to plant extracts was keenly provided in the previous Office action. The unpredictability of agents for treating wounds is equally unpredictable. Typically, to demonstrate the efficacy of a new wound healing agent; the agent should be capable of healing a wound more rapidly than the natural wound-healing process carried out in epithelial tissues. For example, such an agent would have the capability of augmenting the naturally-occurring wound healing process by mechanisms such as increasing epithelial cell growth or wound adhesion or collagen production. The Specification does not provide any evidence in the form of examples; nor

does the Specification teach that the pawpaw products of the claims provide for any mechanism which would aid in wound healing.

Applicant submits that one of skill in the art reading the specification would understand that the specification enables the breadth of the claims. Further, the supplemental experimental evidence in the attached Exhibits 1 & 2 further supports Applicant's position that the specification enables the uses recited in claims 12, 14, 65, and 67.

The supplemental evidence in Exhibit #1 provides descriptions of a treatment composition named "OPAL A". Applicant submits that OPAL A is a composition made by a process encompassed by claim 1. In particular, the reference entitled "Project Report: Opal Pilot Bioengineering Run" describes OPAL A being made by a process comprising (a) preparing a pulp from one or more fruit(s) and/or vegetable(s); (b) heating said fruit and/or vegetable pulp up to a temperature in the range of about 40°C to 70° C; (c) mixing between about 1 and 40% w/w of a base having a pKa of less than 11 with the heated fruit and/or vegetable pulp of step (b) whilst said pulp has a temperature in the range specified in step (b) to form a mixture; and thereby obtaining the OPAL A topical composition. Page 8, first full paragraph, describes the production of "Paw Paw 6001S (Standard)". This Paw Paw Standard ("Paw Paw S") composition is then described as "OPAL A" in Table 3 on page 11.

Further, the attached clinical study document Exhibit #2 entitled "Case Reports: Participants Using OPAL A Filtrate and Cream" provide several examples of topical application of "OPAL A" being surprisingly effective at treating a variety of skin ulcers and wounds, as encompassed by claims 12, 14, 65, and 67. Accordingly, the attached clinical evidence Exhibit #1 and #2, which describe the therapeutic effects of "OPAL A" as described in the specification

at the time of filing, are substantive supporting evidence showing that claims 12, 14, 65, and 67 were enabled by the specification at the time of filing.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of this rejection.

D. Anticipation Rejection Under 35 USC 102(b)

The Official Action states that claims 6, 20, 63, 64 and 69-70 are rejected under 35 U.S.C. 102(b) as being anticipated by Schmidt, L. Way Down Yonder In The Pawpaw Patch; Intelligencer Journal, Lancaster, Pa. Oct. 02 (1996).

In view of the remarks set forth herein, this rejection is respectfully traversed.

The test for anticipation is whether each and every element as set forth is found, either expressly or inherently described, in a single prior art reference. Verdegaal Bros. v. Union Oil Co. of California, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); MPEP § 2131. The identical invention must be shown in as complete detail as is contained in the claim. Richardson v. Suzuki Motor Co., 9 USPQ2d 1913, 1920 (Fed. Cir. 1989); MPEP §2131. The elements must also be arranged as required by the claim. In re Bond, 15 USPQ2d 1566 (Fed. Cir. 1990).

Claim 1, from which the rejected claims depend, recites **"heating said fruit and/or vegetable pulp up to a temperature in the range of about 40°C to 70° C"** and **"mixing between about 1 and 40% w/w of a base having a pK_a of less than 11 with the heated fruit and/or vegetable pulp of step (b) whilst said pulp has a temperature in the range specified in step (b) to form a mixture."**

The Official Action at pages 7-9 states (emphasis added):

> Schmidt (1996) writing for the Intelligencer Journal reported several recipes made with pawpaw fruit including pawpaw bread made with 1 cup mashed ripe pawpaw fruit (with skin and seeds removed), 1 teaspoon of baking soda and melted margarine (see p. 2).
>
> This bread is deemed to anticipate the composition claims for the following reasons:
>
> Schmidt taught addition of 1 teaspoon of baking soda (sodium bicarbonate) to one cup mashed fruit. One making the bread of Schmidt, adding the baking soda directly to the cup of mashed fruit would have added about 2% of a base having a pKa of less than 11 (i.e., baking soda, also known as sodium bicarbonate). Because the composition claims are product-by-process and because the process uses open language; the process may additionally include addition of auxiliary ingredients as well as the baking steps included by Schmidt to make a pawpaw bread. While Schmidt did not beat (required by claims 63 and 64 which are dependent upon claim 4) the batter prepared for the bread, Schmidt did mix the batter. It is determined that mixing or beating would not have changed the final product to such an extent to render the product novel over Schmidt absent evidence to the contrary. There is no specific time for beating and hence, claim 4 is broad enough to read on beating for a few seconds or less. **It is further determined that heating the pawpaw fruit to the temperatures required by the claims prior to making the bread of Schmidt would not have changed the overall characteristics of the pawpaw bread.**
> ...
> When the claim recites using an old composition or structure and the use is directed to a result or property of that composition or structure, then the claim is anticipated (MPEP 2100 pp. 2113). In the instant case, although the claims state 'topical composition' because the bread is not precluded from being used topically, the bread of Schmidt anticipates the claimed invention.

The Official Action states that Schmidt teaches adding 1 teaspoon of baking soda (sodium bicarbonate) to one cup mashed fruit. Further, at most, Schmidt appears to describe that the sodium bicarbonate is added to and mixed with the fruit at room temperature. In contrast, claim 1 recites "**mixing between about 1 and 40% w/w of a base having a pK_a of less than 11 with the heated fruit and/or vegetable pulp of step (b) whilst said pulp has a temperature in**

the range specified in step (b) to form a mixture." There is no teaching or suggestion in Schmidt that the baking soda is added to the fruit pulp while the fruit pulp is at a temperature of 40°C to 70° C.

The Official Action appears to discount this recited claim feature, and merely provides the following unsupported conclusory statements:

> It is determined that mixing or beating would not have changed the final product to such an extent to render the product novel over Schmidt absent evidence to the contrary.
> ...
> It is further determined that heating the pawpaw fruit to the temperatures required by the claims prior to making the bread of Schmidt would not have changed the overall characteristics of the pawpaw bread.

Applicants submit that the additional **clinical data** (Exhibits #1 and #2) presented above to address the enablement rejection **provides evidence to the contrary**. **The clinical data goes to show that a composition made by the process of claim 1 indeed has unexpected ulcer and wound healing characteristics not taught or predicted in the art, and further suggests that the process of claim 1 and its recited features are critical to attaining these unexpected results**. Therefore, Applicant submits that the recited feature of mixing base "with the heated fruit and/or vegetable pulp of step (b) **whilst said pulp has a temperature in the range specified in step (b) to form a mixture**" is a critical feature of the current claims and is neither taught nor suggested by the cited reference.

Accordingly, Schmidt does not teach all of the recited claim features as required under 35 USC § 102(b), and does not teach or predict the unexpected results obtained using the claimed composition and process.

In view of the foregoing, it is submitted that the cited reference does not render the presently pending claims obvious within the meaning of 35 USC § 102(b). Accordingly, the Examiner is respectfully requested to withdraw this rejection.

E. Anticipation Rejection Under 35 USC 102(b) or 103(a)

The Official Action states that claims 6 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by, or in the alternative, under 35 USV 103(a) as obvious, over Schmidt, L. Way Down Yonder In The Pawpaw Patch; Intelligencer Journal, Lancaster, Pa. Oct. 02 (1996). The Official Action at page 11 states:

> The teachings of Schmidt were discussed above. Schmidt did not teach the pH of the pawpaw bread. However, since the pawpaw bread contained many ingredients with a high pH, the bread probably has a pH within or 'about' 7.5 to about 9.5 absent evidence to the contrary. Further, the adjustment of pH of a food product would have been well-within the purview of the ordinary artisan at the time the invention was made considering that pH is a result-effective variable. The ordinary artisan was well-aware of the palatability changes which took place upon adjustment of pH in food compositions and adjusting the pH of a food composition to suit varying tastes is deemed obvious lacking any unexpected result.

The Official Action concludes that "with the showing of the references, the burden of establishing non-obviousness is shifted to the Applicants."

Applicant traverses the rejection for at least the following reasons.

To establish a *prima facie* case of obviousness, the Examiner must satisfy three requirements. First, as the U.S. Supreme Court held in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007), "a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions. ...it [may] be necessary for a court to look to interrelated teachings of multiple patents; the effects of demands known to the

design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. ...it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does... because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." (*KSR*, 550 U.S. 398 at 417.) Second, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc. v. Chugai Pharm. Co.*, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991). Lastly, the prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

The Reference Does Not Teach or Suggest All Recited Limitations

Applicant submits that claims 6 and 7 are not anticipated or rendered obvious as indicated for at least the reason that the cited reference does not teach or suggest all of the recited claim limitations.

Regarding claims 6 and 7, Schmidt does not teach a process for making a topical composition as recited in claim 1. The arguments recited above in regard to Schmidt anticipating claim 1 are incorporated herein by reference and applied to the rejection of claims 6 and 7. Since Schmidt does not teach or suggest the process recite in claim 1, then Schmidt also does not teach

or suggest a fruit and/or vegetable derived topical composition obtainable from the process of claim 1. Accordingly, Schmidt does not teach or suggest all of the limitations of claim 6 or 7.

Also regarding claim 7, the Official Action admits that Schmidt does not teach the pH of the pawpaw bread. But then alleges (emphasis added):

> **However, since the pawpaw bread contained many ingredients with a high pH, the bread probably has a pH within or 'about' 7.5 to about 9.5 absent evidence to the contrary.**

The Official Action does not indicate which, if any, of Schmidt's ingredients, other than sodium bicarbonate, have a "high pH", and merely concludes that "many ingredients" have a high pH.

The Applicant submits that one of ordinary skill in the art would understand that the pH of the pawpaw bread described by Schmidt would **not** be within or 'about' 7.5 to about 9.5. The pH of bread is known in the art to be below 7.0. For example, Applicant's response dated February 7, 2008, provided evidence in Exhibit #2 showing the US FDA listing of "Approximate pH of Foods and Food Products". **The pH of every variety of "bread" listed by the FDA falls within the pH range of 5.00 to 6.53.**

Furthermore, Applicant submits that Schmidt's pawpaw bread recipe does **not** contain "many ingredients with a high pH". In fact, it appears that only one ingredient in Schmidt's pawpaw bread recipe has a "high pH", baking soda. Schmidt's bread recipe lists flour, baking soda, salt, margarine, sugar, eggs, mashed pawpaws, milk, lemon juice, and chopped nuts. The FDA pH Listing indicates that eggs (6.58), milk (6.4-6.8), lemon juice (2.0-2.6), and chopped nuts (walnuts 5.42) are all below pH 7. Nearly all fruits on the list, and most vegetables, have a pH less than 7. Sugars are generally considered acidic, such as for, example honey (3.7-4.2). A

brief review of the literature indicates that both flour and margarine or butter have a pH less than 7. For example, US Patent No. 5,560,953, FIG. 5, indicates that untreated and treated flour has a pH significantly less than 7. Clearly, in view of this evidence, the pH of the Schmidt's bread will likely be less than 7. Contrary to the assertions made in the Official Action, Schmidt does **not** teach or suggest a fruit and/or vegetable derived topical composition having a pH in the range of about 7.5 to about 9.5 as recited in claim 7.

Accordingly, for the reasons provided above, Applicant submits that Schmidt does not teach or suggest all of the recited features of claims 6 and 7.

For at least this reason, Applicant submits that claims 6 and 7 and not anticipated under 35 USC 102(B), nor rendered *prima facie* obvious under 35 USC 103.

No Motivation To Modify the Reference And No Reasonable Expectation of Success

Applicant submits that one of ordinary skill in the art would not have been motivated to adjust the pH range of Schmidt's bread recipe to a pH ranging from about 7.5 to about 9.5, and neither would there have been a reasonable expectation of successfully doing so.

The Official Action alleges (emphasis added):

> Further, the **adjustment of pH of a food product would have been well-within the purview of the ordinary artisan** at the time the invention was made considering that pH is a result-effective variable. The ordinary artisan was well-**aware of the palatability changes which took place upon adjustment of pH in food** compositions and **adjusting the pH of a food composition to suit varying tastes is deemed obvious** lacking any unexpected result.

Adjustment of pH in food compositions might be used in some instances to affect the palatability of food compositions and to suit varying tastes of one eating the food. However, Applicant submits that the adjustment of pH of food compositions generally involves adjusting

the food composition to lower pH values, and in particular to acidic pH values, or to pH values that are less acidic but still significantly less than pH 7.5. In part, lowering the acidity of food is preferred in order to maintain food safety by inhibiting the growth of microorganisms (See Exhibit #3, submitted on February 7, 2008). Furthermore, the pH of all six varieties of "bread" on the FDA list have a pH ranging from 5.00 to 6.53. Flavor enhancers listed on the FDA list are generally low acid or high acid. Adding more or less of particular acidic flavor enhancer such a vinegar, lemon juice, fruit juice, fruits, honey, soy sauce, etc. will likely still maintain the pH of bread well below a pH of about 7.5. Applicant submits that one of ordinary skill in the art reading Schmidt and this FDA list would not be motivated to modify the Schmidt bread recipe to have a resulting pH ranging from about 7.5 to about 9.5, and neither would there have been a reasonable expectation of successfully doing so.

Applicant respectfully submits, that for at least this reason as well, there is no *prima facie* case of obviousness.

The Claimed Subject Matter Provides An Unexpected Result

However, assuming *in arguendo,* that each of the recited claim features were taught or suggested by the cited reference, and that there is a *prima facie* case of obviousness, Applicant respectfully submits that the present claims provide an unexpectedly superior improved composition that unexpectedly provides for ulcer and wound healing. In this regard, Applicant respectfully draws the Examiner's attention to the clinical data in the attached Exhibits #1 and #2 showing that the topical administration of a composition (OPAL-A) encompassed by claim 6 and 7, and made by a process encompassed by claim 1, results in the unexpected ability to heal ulcers

and wounds. The cited reference does not teach or suggest this improved composition nor its ability to unexpectedly provide ulcer and wound healing.

Accordingly, for any of the above reasons, Applicants respectfully submits that the presently claimed subject matter is non-obvious.

F. Obviousness Rejection Under 35 USC 103(a)

The Official Action states that Claims 1-7, 12, 14, 20, 23-25, 27, 30, 32, 63-65 and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over (1) **Burchard**, H., The Washington Post, (1999) in view of (2) **Purdue University** Online (2001); (3) US Patent No. 1,997,616 to **Wallerstein**, L. (1935); (4) **Blackstone**, R., A Gift To Avoid The Yule Budget Jam; The Province, Vancouver, B.C. (1993); (5) **Schmidt**, L. Way Down Yonder In The Pawpaw Patch; Intelligencer (1996); (6) and **Burckhardt**, A. Quick Pickles And Jams; Minneapolis Star and Tribune (1986).

The Official Action states (emphasis added):

> **Burchard, H.** reporting for The Washington Post (1999) taught that pawpaw, known botanically as Asimina triloba, was an endogenous, fruit-bearing plant of the Eastern United states as well as Canada, often harvested for it's edible fruit as well as the fruit's medicinal and insecticidal properties (see pp. 1-2). Burchard indicated pawpaw fruit to be used in various food items such as milkshakes, baked goods, pina coladas, pie, ice cream, chutney and brandy. Burchard specifically disclosed a recipe for pawpaw fruit jam which included combining pawpaw pulp, water, applesauce, apple juice, lime juice, sugar and pectin, stirring, bringing to a boil (100 °C), 'stirring constantly' and pouring into jelly jars (see entire reference, especially page 4).
>
> Burchard did not specifically teach wherein pawpaw fruit jam was mixed with about 1 to 40% or 3-14% of a base having a pKa of less than 11 (Applicant's elected species is sodium bicarbonate), filtering, beating, freezing and thawing prior to filtering, wherein the fruit pulp was heated to about 40 °C to 70° or from about 50° C to 60 °C.

Purdue University Online (PUO)(archived to 2001) teaches that pawpaw; known by its botanical name of Asimina triloba, was well known for its edible fruit; used as a food source containing larger amounts of vitamins, minerals, amino acids and food energy than apple, peach or grape (pp. 1-2). PUO reported that the pawpaw fruit are useful for making food products such as blended fruit drinks, baby food, ice cream, puree and frozen food products. PUO additionally noted the cosmetic potential of the pawpaw fruit (p. 2).

Fruit jellies are often made with the addition of bicarbonates in order to strengthen the jelly compositions and to provide longer shelf-life according to **Wallerstein** (US 1,997616). Wallerstein teaches the addition of 1 part of magnesium carbonate (magnesium bicarbonate- MgCO3) to 30 to 50 parts of pectin (see p. 1, col. 2). (pectin compositions are added to fruit/fruit juices to produce jellies and jams).

Blackstone, reporting for The Province. Vancouver, B.C.: Nov 17, 1993. pg. B.8, pp. 1-2 of ProQuest indicated that the fruit used in jams could be heated prior to addition of pectin and sugar; and thereby the fruit was heated prior to boiling (see p. 1).

Schmidt, L. reporting for the Intelligencer Journal of Lancaster, PA, reported several recopies using pawpaw fruit including a pawpaw bread and zabaglione and ice cream; wherein the seeds and peel were removed prior to use (see pp. 2-3).

...

Hence, the steps for producing the pawpaw composition as claimed is deemed obvious considering that pawpaw fruits are well-known in the art to be an edible fruit which is prepared into food compositions such as jams. The alterations of temperature and amounts of bicarbonate in the claim are deemed within the level of the ordinary artisan at the time the invention was made in order to vary these parameters under normal working conditions to prepare pawpaw jams and jellies: "[a] person of ordinary skill is also a person of ordinary creativity, not an automaton KSR 127S. Ct. at 1742. Hence, absent any unexpected results, these variations are not deemed patentable over the prior art teachings.

Applicant traverses the rejection for at least the following reasons.

To establish a *prima facie* case of obviousness, the Examiner must satisfy three requirements. First, as the U.S. Supreme Court held in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007), "a court must ask whether the improvement is more than the predictable

use of prior art elements according to their established functions. ...it [may] be necessary for a court to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. ...it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does... because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." (*KSR*, 550 U.S. 398 at 417.) Second, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc. v. Chugai Pharm. Co.*, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991). Lastly, the prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

The Reference Does Not Teach or Suggest All Recited Limitations

Applicant submits that claims 1-7, 12, 14, 20, 23-25, 27, 30, 32, 63-65 and 68 are not anticipated or rendered obvious as indicated for at least the reason that the cited reference does not teach or suggest all of the recited claim limitations.

In this regard, the Official Action asserts (emphasis added):

> Although **the prior art does not specifically teach heating the fruit to the specific temperatures as required by the claims** ordinary artisan would have had a reasonable expectation that heating pawpaw to these temperatures would have enabled any additionally added sugar to dissolve into a pawpaw jelly/jam

composition. Further, **it is clear from the prior art that jams were prepared by first heating fruit at a temperature below boiling prior to boiling to dissolve the sugar and pectin into the fruit**. Hence, the **choice of temperature ranges as claimed are deemed within the purview of the ordinary artisan** at the time the invention was made in order to heat the fruit to homogenize the fruit mixture and could have been achieved through routine experimentation. It is clear that the prior art taught heating the fruit composition prior to making jams/jellies.

The **ordinary artisan would have had a reasonable expectation of success in producing a pawpaw jam with the addition of a bicarbonate as taught by Wallerstein in order to increase the stability/shelf life** of a pawpaw jelly or jam.

It has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. In re Aller, 220 F2d 454,456,105 USPQ 233; 235 (CCPA 1955). see MPEP § 2144.05 part II A. It would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to determine all operable and optimal concentrations of bicarbonate, because this component was an art-recognized result-effective variable which would have been routinely determined and optimized in the food art. Further, if there are any differences between Applicant's claimed method and that suggested by the combined teaching of the prior art, the differences would be appear minor in nature.

The combination of references do not teach or suggest all of the recited limitations of the independent claims, or claims dependent thereon. For example, the combination of references do not teach or suggest mixing "a base having a pKa of less than 11 with the heated fruit and/or vegetable pulp of step (b) whilst said pulp has a temperature in the range specified in step (b) to form a mixture; and thereby obtaining a topical composition" as recited in claim 1. Also, for example, the combination does not teach or suggest mixing the base with the heated pulp while the pulp is at "a temperature in the range of about 40°C to 70° C."

Wallerstein describes using magnesium carbonate to obtain the desired clear and transparent high strength jelly. Wallerstein describes that after the mixture of fruit and magnesium carbonate is prepared "the mixture may then be heated or boiled, preferably with

slight stirring." Page 2, col. 1, lines 21-23. Wallerstein obtained a "substantial decrease in jelly strength" and "not fully satisfactory" uniformity when magnesium carbonate was replaced with sodium bicarbonate. Page 2, col. 1, lines 49-73, and table on page 2. Thus, Wallerstein's results suggest that it was the magnesium ion which is the critical to achieving the desired properties and not necessarily the carbonate alone. There is no teaching or suggestion to heat the fruit composition prior to adding the base. Furthermore, there appears to be no criticality in Wallerstein's method to add the base only after the fruit pulp is heated to a temperature in the range of about 40°C to 70° C.

The Official Action asserts that "it would have been obvious to one of ordinary skill in the art at the time Applicants' invention was made to determine all operable and optimal concentrations of bicarbonate, because this component was an art-recognized result-effective variable which would have been routinely determined and optimized in the food art." However, one of ordinary skill in the art reading Wallerstein with the combination of references would have determined that all operable and optimal concentrations of bicarbonate would not have included requiring the heating of the fruit pulp to a temperature in the range of about 40°C to 70° C and only then mixing in the base at that temperature to obtain the unexpectedly useful topical composition of the present claims.

Accordingly, Applicant submits that the combination of references do not teach or suggest all of the recited limitations of the present claims. For at least this reason, Applicant submits that the claims are not *prima facie* obvious under 35 USC 103.

The Reference Does Not Teach or Suggest All Recited Limitations of Claim 5

Regarding claim 5, the Official Action alleges that the recited features are not given patentable weight (emphasis added):

> With regard to claim 5 which states "**wherein the mixture obtained in step (c) has a pH in the range of about 7.5 to about 9.5**," Applicant is directed to MPEP § 2111.04:
> The determination of whether each of these clauses is a limitation in a claim depends on the specific facts of the case. In Hoffer v. Microsoft Corp., 405 F.3d 1326, 1329, 74 USPQ2d 1481, 1483 (Fed. Cir. 2005), the court held that when a **"whereby' clause** states a condition that is material to patentability, it cannot be ignored in order to change the substance of the invention." Id. However, the court noted (quoting Minton v. Nat 'I Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003)) that a "whereby clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited:" Id.< (emphasis added).
> Hence, it follows from Minton v. Nat 'I Ass 'n of Securities Dealers, Inc., 336 F.3d 1373, 1381, 67 USPQ2d 1614, 1620 (Fed. Cir. 2003) that **claim 5 simply 'expresses the intended result of a process step'** and is thus not given patentable weight.

Applicant respectfully disagrees with the application of MPEP 2111.04 to assert that the recited wherein clause does not carry patentable weight. MPEP 2111.04 expressly states (emphasis added): "**Claim scope is not limited by** claim language that suggests or makes optional but does not require steps to be performed, or by **claim language that does not limit a claim to a particular structure.**" Applicant submits that the recited phrase "wherein the mixture obtained in step (c) has a pH in the range of about 7.5 to about 9.5" specifically structurally limits the claim to a composition having the specified pH range. The composition obtained from step (c) is not merely an intended result of a single process step, but is the final composition obtained by the claimed process. Furthermore, a composition having a specified pH range very clearly has a very specific limit on its structure. Compositions not having the hydrogen ion concentration indicated by the recited pH range are not encompassed by the claim,

and therefore the recited feature very clearly limits the scope of the claim. Accordingly, the recited wherein clause very clearly limits the structural scope of claim 5.

In this regard, the combination of references do not teach or suggest a process for making a fruit and/or vegetable composition having a pH ranging from about 7.5 to about 9.5. In fact, the combination of references were selected from the food and jelly making arts which generally require or result in compositions having a very low pH for food compositions containing a significant amount of fruit. For example, Wallerstein's use of a base with their composition still results in a jelly composition having a pH of between about 3.0 to 3.4. Page 2, col. 1, lines 67-70. The various food products made by the other combined references would also likely result in compositions having a pH generally less than 7.5. For example, see the FDA Listing of Food pH cited above.

Moreover, the food art is often not concerned about the resulting pH of any particular recipe, except for foods requiring low pH for safely preserving and storing certain products, such as described in Wallerstein, and as required by the FDA in the preparation and preservation of certain foods. (See Exhibit #3 in the Response dated February 7, 2008, CFR regarding FDA regulations for acidifying foods for safety.) However, the present claimed subject matter is not properly categorized as a "food art" but is more closely related to the pharmaceutical arts or topical products arts where resulting pH is commonly recognized as a critical structural feature of many compositions. Accordingly, the Official Action might be placing too much emphasis on examining the claimed subject matter in view of the food arts. The claimed subject matter is not a food composition or food process, but is appropriately categorized in the pharmaceutical or topical arts. The claims clearly recite that the obtained composition is a topical composition.

Moreover, one of ordinary skill in the art reading the specification would understand that the subject matter is related to a topical or pharmaceutical composition, and is not directed to palatability of compositions or nutritional value of compositions.

To conclude, Applicant submits that the "wherein clause" of claim 5 provides a structural limitation of the resulting composition that is commonly recognized as a potential patentable distinction in the pharmaceutical and topical arts. The combination of cited references from the food arts describe recipes for making food compositions that are generally understood to have a pH significantly lower than 7.5. The Wallerstein reference, which is used to show that a base can be mixed with a fruit composition, teaches that their method will result in a pH ranging from about 3.0 to 3.4 which is known to be required for safety in the jelly and fruit preserves arts. In contrast, the combination of references do not teach or suggest a process of making a topical composition having a pH in the range of about 7.5 to about 9.5 as required in claim 5.

Accordingly, Applicant also submits that, for these additional reasons, the combination of references do not teach or suggest all of the recited limitations of claim 5.

No Motivation To Combine the Established Functions of the References and No Reasonable Expectation of Successfully Combining Them

In arguendo, even if the combination of references do teach or suggest all of the recited claim limitations, Applicant submits that one of ordinary skill in the art would not have been motivated to combine the established functions of the cited references to obtain a method requiring heating of the fruit pulp to a temperature in the range of about 40°C to 70° C and only then mixing in the base at that temperature to obtain the unexpectedly useful topical composition

of the present claims. Furthermore, there would have been no reasonable expectation of successfully combining the references to obtain the claimed subject matter.

The Official Action states (emphasis added):

> The Supreme Court has acknowledged that:
> When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. **If a person of ordinary skill can implement a predictable variation**..103 likely bars its patentability...if a technique has been used to improve one device, and a **person of ordinary skill in the art would recognize that it would improve similar devices in the same way**, using the technique is obvious unless its actual application is beyond that person's skill. **A court must ask whether the improvement is more than the predictable use of prior-art elements according to <u>their established functions</u>**...
> ...the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results (see KSR International Co. v. Teleflex Inc., 82 USPQ2d 1385 U.S. 2007) emphasis added.
>
> From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

A KSR analysis might include attempting to combine "the predictable use of prior-art elements [of Wallerstein, PUO, et al.] according to their established functions". Wallerstein's alleged <u>established function</u> is using a method of mixing base with fruit pulp to obtain a jelly of improved strength and consistency having improved commercial value among fruit jellies. Page 1, col. 1, lines 1-28. **The Purdue University Online (PUO)'s alleged <u>established function</u>** might be that pawpaw has cosmetic potential. One of ordinary skill in the art would not look at all to Wallerstein's method of improving the commercial value of fruit jellies to provide an improved topical composition. Furthermore, one of ordinary skill in the art would not look to Wallerstein to provide an improved topical composition wherein the mixing of

a base with a fruit pulp would be expected to result in a topical composition having enhanced jelly-like strength and improved commercial value among jellies. **Accordingly, following the KSR analysis identified in the Official Action, one of ordinary skill in the art would not find it obvious to combine the predictable use of prior-art elements of Wallerstein and PUO, and the other references, according to their established functions to obtain the claimed subject matter.** Thus, a KSR analysis indicates that it would not have been obvious to combine the cited references to obtain the claimed subject matter.

In addition, there would also have been no reasonable expectation of successfully combining Wallerstein's method of enhancing the strength of jelly with PUO's suggestion to use pawpaw as a cosmetic to obtain an process for making a fruit and/or vegetable derived topical composition comprising the steps of: a) preparing a pulp from one or more fruit(s) and/or vegetable(s); b) heating said fruit and/or vegetable pulp up to a temperature in the range of about 40°C to 70° C; c) mixing between about 1 and 40% w/w of a base having a pK_a of less than 11 with the heated fruit and/or vegetable pulp of step (b) whilst said pulp has a temperature in the range specified in step (b) to form a mixture; and thereby obtaining a topical composition. One of ordinary skill in the art attempting to combine the established functions of Wallerstein's method with PUO's suggestion for a pawpaw cosmetic would not have had a reasonable expectation of successfully obtaining the presently claimed process of making a topical composition.

Accordingly, Applicant submits that under a KSR analysis, one of ordinary skill in the art would not have been motivated to combine the established functions of the prior art references to obtain the claimed subject matter. Similarly, one of ordinary skill in the art would not have had a

reasonable expectation of successfully combining the established functions of the prior art references to obtain the claimed subject matter. For at least either of these reasons, Applicant submits that the claims are not *prima facie* obvious under 35 USC 103.

The Claimed Subject Matter Provides An Unexpected Result

However, assuming *in arguendo,* that each of the recited claim features were taught or suggested by the cited reference, and one could have combined the established functions of the references to arrive at the claimed subject matter, and that there is a *prima facie* case of obviousness, Applicant respectfully submits that the present claims provide an unexpectedly superior improved composition that unexpectedly provides for ulcer and wound healing. In this regard, Applicant respectfully draws the Examiner's attention to the clinical data in the attached Exhibits #1 and #2 showing that the topical administration of a composition (OPAL-A) encompassed by the claims, and made by a process encompassed by claim 1, results in the unexpected ability to heal ulcers and wounds. The cited reference does not teach or suggest this improved composition nor its ability to unexpectedly provide ulcer and wound healing.

Accordingly, for any of the above reasons, Applicants respectfully submits that the presently claimed subject matter is non-obvious.

G. Comments On The Term "Pawpaw"

Applicant submits that the term "pawpaw" as recited in the specification, and some dependent claims, is a term of art that is commonly used to clearly and definitely refer only to tropical *Carica* species, such as *Carica papaya.*

1. "Pawpaw" is a Term of Art Used Worldwide

Applicant submits that tropical *Carica* species, such as *Carica papaya*, is commonly referred to as "pawpaw" worldwide, and that the term "pawpaw" as used in the specification is a known term of art that refers to *Carica* species. For every instance where the term "pawpaw" is used in the specification, the term is used to refer to *Carica* species. For example, Applicant submits that all of the "pawpaw" examples discussed in the specification employed *Carica* species fruit. Moreover, Applicant submits supplemental evidence herein showing that the term "pawpaw" is a clear and definite term of art known worldwide for describing *Carica* species, common in tropical and sub-tropical areas worldwide. This is supported by the attached expert's declarations from pawpaw *Asimina* expert Neal Peterson.

Applicant notes that in parts of North America, the term "pawpaw" can at times be used to refer to temperate North American trees and fruit of the genus *Asimina*, including *Asimina triloba*, indigenous to temperate forests of eastern United States and Canada. The origin of the term "pawpaw" for *Asimina* species is unknown, though some believe the term may have originated in the early 17th Century with John Smith's founding of Jamestown Virginia where the settlers may have thought the unknown fruit of *Asimina* was a *Carica* species, which was known to the Spanish and the English in Cuba and the Caribbean islands at that time. Furthermore,

English speakers at that time already had been commonly referring to *Carica* species as "pawpaw". Pawpaw *Asimina* species are unique in that they are the only temperate members of the *Annonacaea* family, and no other members of the *Annonacaea* family have been called "pawpaw". Further, Pawpaw *Asimina* species require approximately 400 hours of chilling to break dormancy, and thus will not easily grow in tropical or sub-tropical zones (*e.g.*, near the Gulf of Mexico) where extended cold periods are rare or non-existent.

Applicant also submits herewith an Information Disclosure Statement which cites various references indicating the widespread use of the term "pawpaw" for *Carica* species, including the following evidence.

2. Cookbooks and Recipes

Applicant submits that in cookbooks and recipes originating outside of the United States which use the *Carica* species as an ingredient, the *Carica* species is widely and consistently referred to as "pawpaw".

For example, the recipe entitled "Prawn, Pawpaw, and Chili Salad" is found at page 75 of "Curry and Chili Cookbook", 3rd edition, eds. Moss et al., Bay Books, NSW Australia, (2008) (cited in the attached IDS) and describes the use of "pawpaw". There is no mention of "papaya" or "*Carica*" in the cookbook. The upper photograph shows a slice of the fruit of the *Carica* species. Accordingly, a person of ordinary skill in the art would understand that the term "pawpaw" is used consistently in this recipe to clearly and definitely refer only to the fruit of the *Carica* species.

A recipe for Biriani (a rice dish), originating from the coast of tropical Kenya , is found at http://nutford.kijabe.org/recipes.html, and cited in the attached IDS. One of the ingredients is an "unripe pawpaw." As indicated in the attached expert's declaration by Neal Peterson: the term "pawpaw" used in the recipe would not be confused with the *Asimina* "pawpaw" species because (1) the recipe originates from the tropical coastal region of Kenya which commonly uses the British English term "pawpaw" only to refer to Carica species; (2) *Asimina* species do not exist in Kenya; (3) in our expert's experience, persons from tropical regions of Africa are generally not aware of *Asimina* "pawpaw" species; and (4) persons familiar with using and cooking *Asimina* fruit would not suggest cooking unripe *Asimina* "pawpaw" fruit. Accordingly, this African recipe is consistent with the understanding that the term "pawpaw" is widely recognized by non-American English speakers around the world as the common term for clearly and definitely describing *Carica* species.

Similarly, recipes using the term "pawpaw" to exclusively refer to *Carica* "pawpaw" fruit are also found in a variety of widely dispersed regions, including, for example, the Caribbean, India, South Pacific, Australia, South Africa, and Great Britain. For example, recipes from the Caribbean in tropical America also commonly use the term "pawpaw" to exclusively refer to *Carica* "pawpaw" fruit as exemplified at http://www.recipe.dominica-weekly.com/?s=pawpaw (cited in the attached IDS).

3. Technical Publications And Databases

Further, in English-language technical publications and databases originating outside of the United States which provide information on the *Carica* species, the *Carica* species is widely and consistently referred to as "pawpaw".

For example, the United Nations' Food and Agriculture Organization (UN-FAO) indicates that the term "pawpaw" is widely recognized worldwide as referring to *Carica* species. The UN-FAO Information Network on Post-Harvest Operations (INPHO) Compendium assembles and disseminates information of paramount importance regarding the world's staple foods and edible crops. In regard to *Carica* species, Applicant notes that UN-FAO-INPHO Compendium Chapter XXII is entitled "Pawpaw" (authored by J. De La Cruz Medina et al. of The Technology Institute of Veracruz, Mexico). This *Carica* species chapter begins at http://www.fao.org/inpho/content/compend/text/CH22_01.htm. (Submitted herewith in the accompanying IDS.) The term "Pawpaw" is used throughout the text, Tables, and Figure Legends to refer to *Carica* species. This UN report is consistent with the understanding that the term "pawpaw" is widely recognized by non-American English speakers around the world as the common term for clearly and definitely describing *Carica* species.

As a further example, a "pawpaw" information sheet is published by The Royal Botanic Gardens (Kew Gardens) of Great Britain which provides information sheets on numerous plants at their website. The Royal Botanic Gardens, the central botanical authority of Great Britain and the British Commonwealth, uses the term "pawpaw" in its hyperlink and throughout its published pawpaw information sheet found at http://www.kew.org/ksheets/pawpaw.html to refer to the *Carica* species. A copy is submitted with the IDS herewith. This pawpaw information sheet

issued by the central botanical authority of Great Britain and the British Commonwealth, is consistent with the understanding that the term "pawpaw" is widely recognized by non-American English speakers, in particular British English speakers, in both temperate and tropical regions around the world, as the common term for clearly and definitely describing *Carica* species.

Clearly, based upon *Carica* species technical publications and databases originating outside of the United States, horticultural experts understand that the term "pawpaw" is widely used outside of the United States to commonly, clearly, and definitely refer to only *Carica* pawpaw species throughout much of the British English speaking world.

4. Accepted Usage of the Term "Pawpaw" Worldwide

The evidence indicates that British Commonwealth English-speakers understand that the term "pawpaw" is used to commonly, clearly, and definitely refer only to *Carica* species throughout much of the British English speaking world. Further, the evidence also indicates that many British Commonwealth English-speakers understand that the terms "pawpaw" and "papaya" are both used to commonly, clearly, and definitely refer only to *Carica* species throughout much of the British English speaking world.

In general, British Commonwealth English-speakers residing outside of North America are quite often not aware of the less common temperate North American "[*Asimina*] pawpaw" species. Accordingly, among English-speakers residing outside of North America, the use of the term "pawpaw" for *Carica* species is unquestionably both clear and definite. Residents of the Pacific Islands, Polynesia, New Zealand, Australia, Central America, South America, Africa, India, and elsewhere understand that "pawpaw" refers clearly and definitely to *Carica* species.

As "pawpaw" (*Carica*) moved throughout the British Commonwealth it continued to be referred to as "pawpaw".

As indicated in the attached Peterson Declaration, it is commonly found that many English-speakers residing outside of North America are not aware that *Asimina* "pawpaw" species native to temperate North American even exist, let alone that the term "pawpaw" can refer to such species. Our expert often receives correspondence from English-speakers residing outside of North America who use the term "pawpaw" solely to refer to *Carica* species. For example, our expert commonly receives e-mail or letters from persons in tropical or sub-tropical zones outside of the United States who are contacting Peterson Pawpaws to obtain information about "pawpaws" or to purchase "pawpaw" trees. However, with most of these inquiries from tropical areas, the correspondents are inquiring about *Carica* species. In these instances, the correspondents are clearly using the term "pawpaw" solely to refer to *Carica* species, and appear to be totally unaware that the term "pawpaw" is commonly used in the United States to refer to the unrelated North American *Asimina* species. In general, it is our expert's experience that among English-speakers residing outside of North America, the use of the term "pawpaw" is commonly used to clearly and definitely refer to the *Carica* pawpaw species. Similarly, it is also our expert's general experience that non-American English-speakers residing in tropical and sub-tropical regions are commonly not aware that the term "pawpaw" can be used in the United States to refer to the less common temperate North American *Asimina* species.

5. MPEP and CFR Requirements Regarding Use of American English

In addition, in regard to the use of British English terms in patent applications, the Applicant points out that the MPEP and CFR only require that a US patent application be in the English language, and there is no additional requirement that the English must be American English. MPEP 608.01 states (emphasis added):

> Examiners should not object to the specification and/or claims in patent applications merely because applicants are using British English spellings (e.g., colour) rather than American English spellings. It is not necessary to replace the British English spellings with the equivalent American English spellings in the U.S. patent applications. Note that 37 CFR 1.52(b)(1)(ii) only requires the application to be in the English language. **There is no additional requirement that the English must be American English.**

While MPEP 608.01 specifically refers to British English spellings, Applicant submits that there is also no additional requirement that the English definitions must be American English definitions and not British English definitions. Also, where a term has achieved meaning as a term of art, there should be no additional requirement that the English term of art must be an American English term of art and not a British English term of art.

6. Conclusion Regarding Usage of the Term "Pawpaw" in the Specification

Accordingly, for reasons discussed herein, Applicant submits that the term "pawpaw" used in the specification was widely recognized at the time of filing as clearly and definitely referring to *Carica* species. This conclusion is supported with the various submitted evidence filed herewith, including the various cited references and two expert declarations under 37 CFR 1.132.

Moreover, the Applicant submits that they should not be denied the ability to claim subject matter on the basis that the term of art "pawpaw", as used at the time of filing to refer to *Carica* species, has acquired a different meaning in a different English-speaking region, the United States, or among American-English speakers in general. To deny the Applicant the right to pursue subject matter defined using a worldwide recognized non-American English term of art would deny the Applicant the equal right to pursue patent protection afforded to those Applicants who are permitted to rely on other non-American English terms of art.

II. CONCLUSION

In view of the foregoing, Applicant respectfully requests the Examiner to reconsider and withdraw the rejections of the claims and to allow the pending claims.

If the Examiner has any questions or wishes to discuss this matter, the Examiner is welcomed to telephone the undersigned attorney.

Respectfully submitted,

THE NATH LAW GROUP

Date: April 16, 2010

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EXHIBIT #1 - 20 pages

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PROJECT REPORT:

OPAL PILOT BIOENGINEERING RUN

PREPARED FOR PHOENIX EAGLE PTY LTD

EXECUTIVE SUMMARY

An Opal bioengineering run was performed on the 5th - 16th of June 2006 for the purpose of assessing the proposed bioprocess schema outlined on the Progress Report prepared by Q-Gen and supplied to Phoenix Eagle Company Pty Ltd on the 4th of May 2006. The objective for this run was also to provide Opal samples to collaborators of Phoenix Eagle Company Pty Ltd for testing and analysis.

The bioengineering run was comprised of four stages of manufacture: preparation of Paw Paw and Peach pulp, preparation of extract from pulp, blending of extracts, and dispensing. The run was completed successfully, with only minor complications that had no apparent detrimental effect on product quality. Bioburden and sterility testing was performed by Q-Gen and further testing, including HPLC analysis and a bioassay, are currently in development.

It is recommended that another bioengineering run be performed prior to phase I clinical lot manufacture to further develop the process.

This report constitutes the final deliverable by Q-Gen Pty Ltd for the contract between Phoenix Eagle Company Pty Ltd and Q-Gen Pty Ltd.

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INTRODUCTION

The primary purpose of performing a bioengineering run was to establish whether the proposed bioprocess schema detailed in the Progress Report prepared for Phoenix Eagle Company Pty Ltd by Q-Gen on the 4th of May 2007 could be accomplished; to determine the suitability of existing equipment, and; to assess requirements for additional equipment and/or the inclusion or amendment of process steps. The secondary purpose was to provide product to collaborators of Phoenix Eagle Company Pty Ltd to use in proteomics and animal studies, and for the development of a bioassay.

The bioengineering run was performed with the aid of draft protocols (refer to Appendix I for copies) by Nicole Bleasdale, Jane Talbot and Allison McLean. Tom McArthur observed the puree and extract preparation processes and we wish to thank him for the guidance and assistance that he provided.

MATERIALS

In-process materials used in the bioengineering run are listed in the following table:

MATERIALS	Q-GEN ID (if applicable)	AMOUNT USED
Paw Paws	NA	25
Peaches - SPC, sliced, in natural juice, 3kg tin	2215	3
Sodium Metabisulfite	2213	2800g
Sodium Bicarbonate	2188	3282g
Silicon Tubing 9.5 x 15.9mm	1798	8m
Flexboy (Stedim) Bags 5L (sterile)	2210	12
MPC Sealing Caps, Female	2211/2212	12
EVA Plasma Component (Stedim) Bags 1L	2219	19
Exchange Couplers	1897	25
Silicon tubing - size 16, peroxide cured	1511	3m
Grape Fruit Seed Extract	2189	9g
Syringe – 10mL	617	1
Vials - 10mL, amber glass, sterile	2203	817
Eye-dropper lids – amber glass, sterile	2200	817

In addition to the above, the following General Consumables were used throughout the process:

- Drapes, sterile
- Gloves, sterile
- Cleanroom Garments, autoclaved
- Face Masks
- Parafilm (sealing tape)

EQUIPMENT

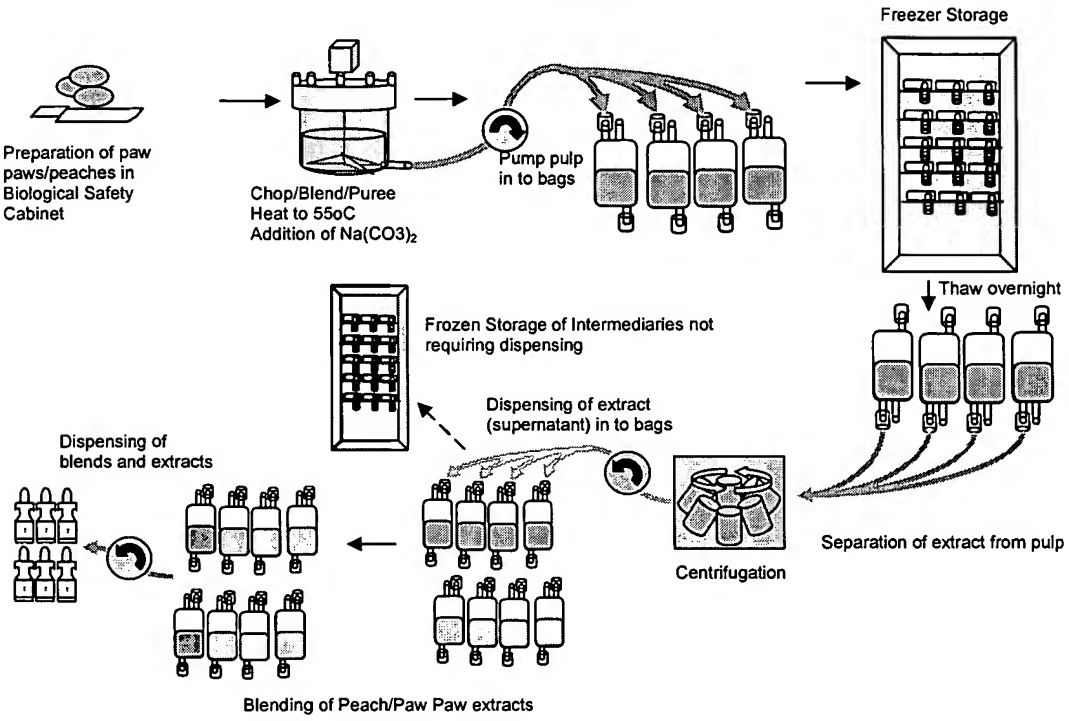
The following table details the equipment used for the bioengineering run:

FACILITIES & EQUIPMENT	ASSET/ SERIAL NO.	CRITERIA USED TO ESTABLISH SUITABILITY
Facilities		
Seed Lots Laboratory (CJ38)		Tested and Certified
Fermentation Laboratory 2 (CJ36)		Tested and Certified
Equipment (used throughout process)		
Class II Biological Safety Cabinet	QIMR6105	Cleaned and set to RUN mode
Preparation of Puree		
20L Bucket		Cleaned
Stainless Steel Bowl		Sterile
Can Opener		Sterile
Knives		Sterile
50L Mixing Tank (with pureeing impeller and motor)	QIMR7743	Sanitised in 20g/L Sodium Metabisulfite for >10mins then dried for > 10mins
ADI1010 Biocontroller and thermocontroller	QIMR6091	NA
50L Fermenter Temperature Probe	QIMR6091	Calibrated
Stainless steel funnel		Clean, sanitised
Digi DI-80 Balance (Max. weight load = 200kg)	QIMR7675	Calibrated
Precisa XT Balance (Max. weight load = 6kg)	QIMR7674	Calibrated
Peristaltic Pump (lge one)	QIMR6754	Clean
Size 16 silicon tubing with 1 ½" hosetail fitting and coldar adaptor (female)		Sterile
ULT (-80°C) Freezer	QIMR5253	Monitored and DAS alarms enabled
Preparation of extracts		
1L PP Centrifuge Bottles with lids		Clean and sterile
Avanti J-20 Centrifuge	QIMR4538	Clean Rotor JLA-8.1000
Masterflex Easy-Load L/S Peristaltic Pump	31876/1	Sterile
Seed Lots Fridge	QIMR4859	Monitored and DAS alarms enabled
Blending/Dispensing		
Flexicon Dispensing Pump	QIMR8127	Clean
Filling Needle		Clean and sterile
Tubing assembly for dispensing pump		Clean and sterile
Filling needle stand		Clean
ULT (-80°C) Freezer	QIMR7641	Monitored and DAS alarms enabled

BIOPROCESSING SCHEMA

The following diagram illustrates the proposed bioprocessing scheme used to partially draft production protocols.

Diagram 1: Bioprocessing Schema



Note: It was agreed that filter sterilisation of the final product would not be performed due to concern that the procedure may remove or reduce the active compound, which to date, has not been identified.

SELECTION OF PAW PAW

Due to Innisfail bearing the direct brunt of Cyclone Larry on the 20th of March 2006, and the resulting destruction of Paw Paw crops in the area, the market supply of the fruit was severely diminished for several months afterwards. Therefore the selection and quality of the fruit at the time of the bioengineering run was of some concern.

Tom McArthur accompanied Margie O-Hara (Q-Gen) to Rocklea Markets (Brisbane) on the 5th of June 2006 to collect 20 Paw Paws that had been reserved upon prior arrangement by Margie. The

condition of these Paw Paws is generally described as "ripe but firm". An additional 5 paw paws were purchased from a Fairfield (Brisbane) fruit shop, these were carefully selected by Tom for colour and texture.

PREPARATION OF FRUIT PULP

Preparation of Paw Paw Pulp commenced at 11:30 on the 5th of June 2006 within the Seed Lots Laboratory (CJ38). The process was observed, in full, by Tom McArthur. This section of the manufacturing process was referred to as batch #6001.

To minimise the introduction of skin-borne contaminants from the fruit to the manufacturing process, Paw Paws were first rinsed in RO water prior to their transfer into Seed Lots and then sanitised in approximately 20L of Sodium Metabisulfite Solution (20g/L) for a minimum of 10 minutes prior. Paw Paws were then transferred two or three at a time into the Biological Safety Cabinet (BSC). Under careful supervision by Tom, the tip and the stem end of each paw paw was removed and discarded. The paw paw was then cut in half length-ways and then each half cut in to crescent segments. The seeds, which were dark brown to charcoal in colour, were gently removed from each segment and discarded. Each segment was cut along the growth line, the region that runs parallel to the skin surface. The lower portion of each paw paw segment containing the skin was discarded. The remaining paw paw segment was cut in to chunks and placed into a sterile container in preparation for transfer to the mixing tank. The cutting procedure is illustrated in the diagram below:

Diagram 2: Procedure used to cut Paw Paws

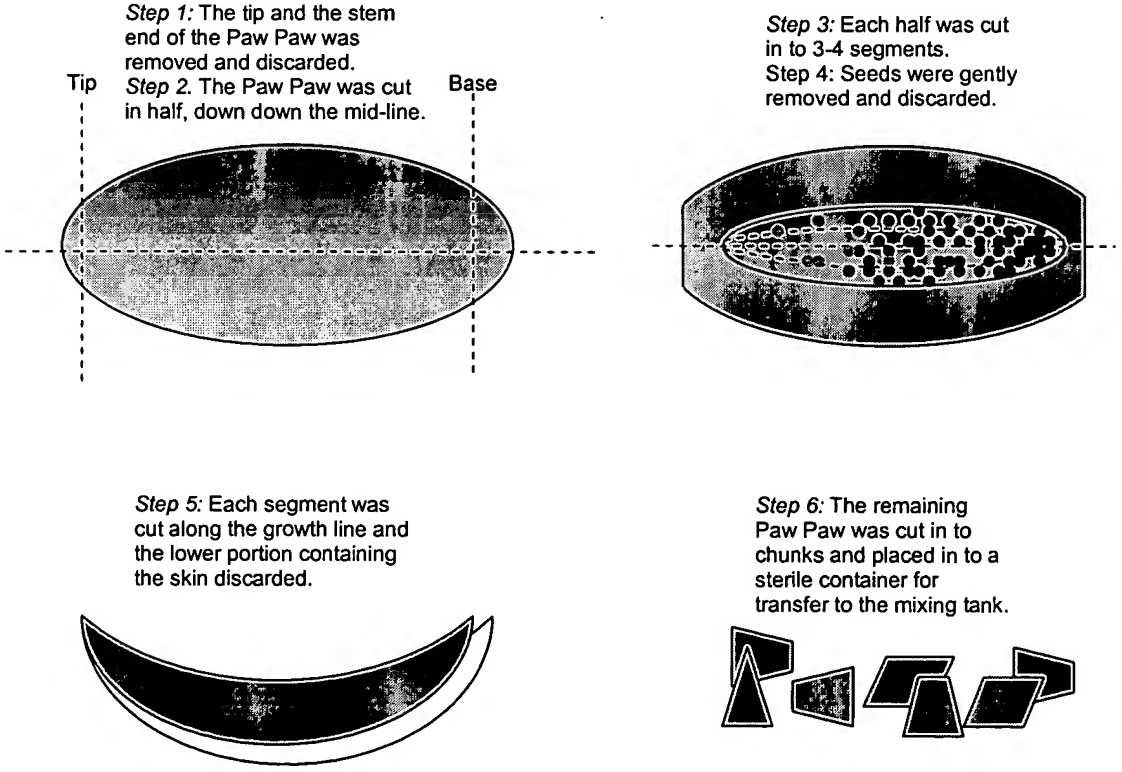
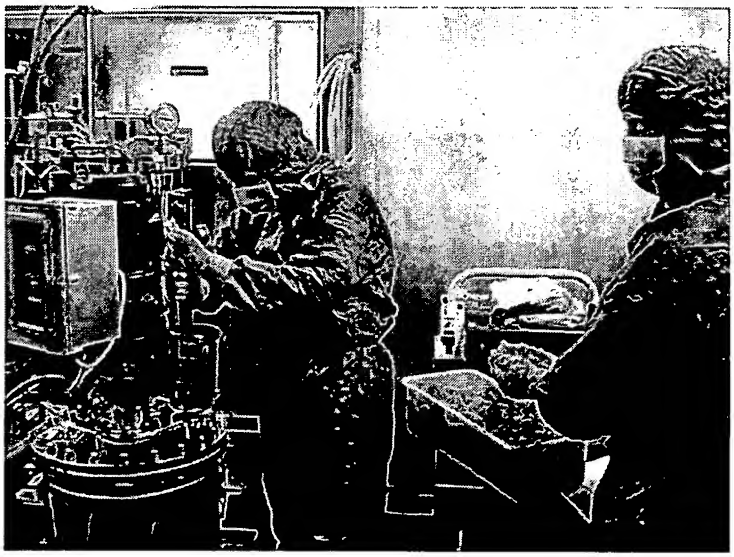


Photo 1: Operators cutting up Paw Paws

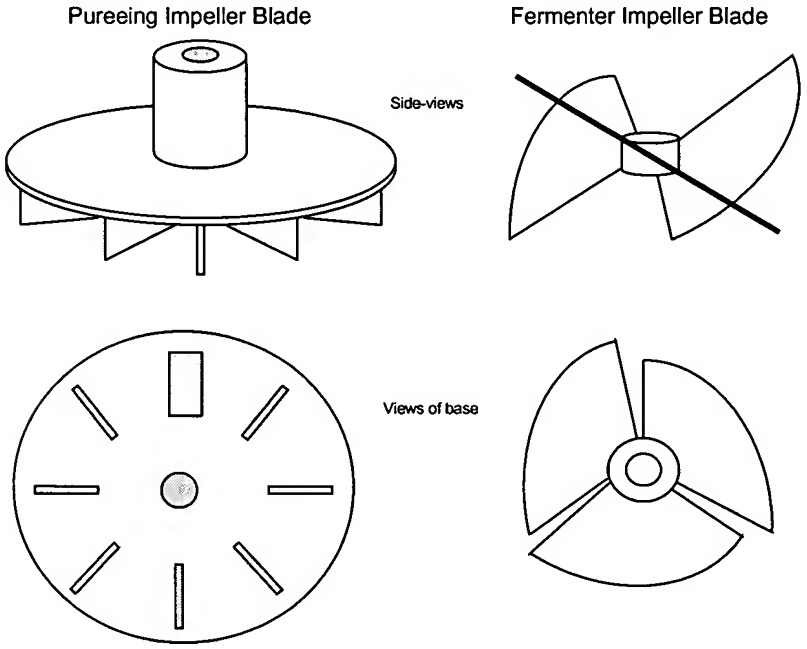
To prepare for the addition of paw paw, the sanitised 50L mixing tank located within Fermentation Laboratory 2 (CJ36) was lifted on to the Digi DI-80 balance. A temperature probe from the 50L fermenter was inserted into the tank head-plate port, the tank jacket connected to the fermenter thermocontroller, and a stainless steel funnel was attached to one of the head-plate ports. The balance was then tared. Paw Paws were transferred into the tank via the stainless steel funnel. Gloved hands and spatulas were needed to push the fruit through the 1" opening. In total, 17840g of paw paw was added to the tank.

Photo 2: Addition of Paw Paw to Mixing Tank



The impeller on the mixing tank was turned on to puree the paw paw. The pureeing impeller consisted of two blades (refer to diagram 1) which was modelled on the design of a domestic stab-mixer. Unfortunately the design proved to be ineffective as the blades were of insufficient diameter and sharpness to puree the paw paw efficiently. After 50mins of use, the decision was made to replace the blades with a single fermenter impeller blade to produce a better vortex and improve sheer force. Nuts and bolts were removed and an attempt was made to lift the head-plate from the tank. Upon failure to dislodge the head-plate, it was discovered that, rather than there being a conventional o-ring seal in place, a rubber gasket had been hot-glued to the lip of the tank. A significant amount of force and leverage was required to separate the head-plate and further effort was required to remove the gasket from each surface. The blades were removed and replaced with the fermenter blade, the headplate reattached to the tank, albeit without any gasket in place, and the nuts and bolts tightened.

Diagram 3: Impeller blades



The pureeing process resumed 75mins later, with greater efficiency. It should be noted that a homogenous mixture was not achieved. Once pureed, sterile tubing consisting of a 1½" hosebarb fitting and a coldar connection was attached to the tank harvest valve. The coldar connection, in turn, was connected to a 5L Flexboy (Stedim) Bag. The harvest valve was opened and 2294g of unheated pulp was transferred to the bag with the use of a peristaltic pump. Once filled and weighed, the bag was disconnected and labelled Paw Paw 6001NH (not heated) and set aside for -80°C storage.

An additional 2421g of unheated paw paw pulp was transferred to a clean beaker. Bicarbonate was added to the pulp to achieve a w/w ratio of 1:9 Sodium Bicarbonate : Paw Paw. 242g of Sodium Bicarbonate was added. This sample was transferred to another 5L bag, labelled Paw Paw 6001NHB (Not heated with Bicarb) and set aside for -80°C storage.

The mixing tank jacket was filled and the thermocontroller set to 55°C. Mixing of the pulp continued during the heating process. The temperature of the tank contents increased slowly but stabilised at

approximately 30°C due to the design of the mixing tank. The tank water jacket does not extend to the base of the tank and therefore only the upper portion of the pulp was directly subjected to heat from the jacket. To increase the temperature further, it was necessary to inject steam directly in to the tank jacket. Steam was injected in bursts to prevent temperature overshoots and stirring continued at high speeds to ensure even temperature distribution. The desired temperature range of 53°C - 60°C was obtained several hours after heating began. Another 5L bag was connected and filled with 2386g of pulp. This bag was labelled Paw Paw 6001H (heated) and set aside to cool prior to transfer to -80°C storage.

The remaining weight of pulp in the tank was calculated to be 10739g. To achieve a w/w ratio of 1:9 Sodium Bicarbonate : Paw Paw, 1074g of Sodium Bicarbonate was added to the tank via the stainless steel funnel. Once added, mixing continued until it was visible that the pulp had stopped effervescing. Once effervescence had ceased, pulp was transferred from the tank in to 5L bags. Four bags were filled, labelled Paw Paw 6001S (standard), bag number, and set aside to cool prior to transfer to -80°C storage. The pH of the cooled pulp standard was pH8.32 at room temperature.

Photo 3: Tom McArthur with Paw Paw Pulp Samples

Once cooled, all samples were transferred to the Freezer Room (CJ10) for -80°C storage at 21:45 on 5th of June 2006. Sample details are recorded below:

Table 1: Paw Paw Pulp Samples

PAW PAW SAMPLE ID	DESCRIPTION	NET WEIGHT (g)
6001NH	Paw Paw Not Heated	2294
6001H	Paw Paw Heated (No Bicarb)	2386
6001NHB	Paw Paw Not Heated with Bicarb	2421
6001S	Paw Paw Standard	Bag#1 2359 Bag#2 2399 Bag#3 2520 Bag#4 2615 Total = 9893g

Preparation of Peach Pulp commenced at 10:40 on the 6th of June 2006 in the Seed Lots Laboratory (CJ38). The process was observed, in full, by Tom McArthur.

Peach tins were transferred in to Seed Lots and sanitised in approximately 20L of Sodium Metabisulfite Solution (20g/L) for a minimum of 10 minutes prior to transfer into the BSC. Using a can opener, tins were opened and peaches and juice emptied into a beaker. A domestic stab-mixer was used to puree the fruit. This method was employed to minimise the process time in light of difficulties experienced during the preparation of the paw paw pulp. The peach pulp was transferred into the tank via the stainless steel funnel. 8960kg of peach was added to the tank.

The mixing tank jacket was filled and the thermocontroller set to 55°C. The pulp was mixed throughout the heating process. The heating methods employed for the paw paw process were used to achieve the desired temperature range of 53°C - 60°C.

To achieve a w/w ratio of 1:9 Sodium Bicarbonate : Peach, 896g of Sodium Bicarbonate was added to the tank via the stainless steel funnel. Once added, mixing continued until the pulp stopped effervescing. Sterile tubing consisting of a 1 ½" hosebarb fitting and a coldar connection was attached to the tank harvest valve. In turn, the coldar connection was connected to a 5L Flexboy bag. The harvest valve was opened and the peach pulp was transferred to the bag with the use of a peristaltic pump. Once filled, the bag was replaced with another, and the process repeated. Five bags were filled, labelled Peach 6001S (standard) and the bags numbered, and set aside to cool prior to transfer to the Freezer Room (CJ10) for -80°C storage at 15:00, 6th of June 2006. Sample details are recorded below:

Table 2: Peach Pulp Samples

PEACH SAMPLE ID	DESCRIPTION	NET WEIGHT (g)
6001S	Peach Standard	Bag#1 1886g Bag#2 1786g Bag#3 1741g Bag#4 1971g Bag#5 1952g TOTAL = 9336g

PREPARATION OF FRUIT EXTRACT

Paw Paw and Peach samples frozen on 5th and 6th of June 2006, respectively, were retrieved from -80°C storage on the 7th of June (pm) and defrosted overnight at room temperature.

Preparation of Paw Paw and Peach Extracts from the defrosted pulp commenced at 09:15 on the 8th of June 2006 within the Seed Lots Laboratory (CJ38). The process was observed, in part, by Tom McArthur. This section of the manufacturing process was referred to as batch #6002.

The defrosted bags were disinfected and placed into the BSC along with centrifuge pots, a balance, and a peristaltic pump. The balance was used to tare the centrifuge pots and for balancing each pair of pots (to prevent centrifuge inbalance). Six centrifuge pots were filled with <1000mL of defrosted fruit puree and then centrifuged at 15900g (8000rpm) for 30 minutes. The speed and length of centrifugation produced a solid pellet and good clarity in the supernatant, however it was felt that a second centrifugation would provide further improvement. In the BSC the supernatant from the initial centrifugation was transferred into new pots and then centrifuged for an additional 20 minutes at 15900g. Upon completion, the pots were returned to the BSC, along with 1L EVA (Stedim) bags and exchange couplers. An exchange coupler was attached to a bag and tubing attached to the exchange coupler. The other end of the tubing was placed in the supernatant. The bag was placed on the balance, tared, and then filled with 900-950mL of supernatant with the use of a peristaltic pump. Once filled, the bag was removed from the exchange coupler, sealed, labelled, and transferred to the Seed Lots fridge for 2-8°C storage. A new bag was fitted to the exchange coupler and the transfer of supernatant continued. This process was repeated until all supernatant from the centrifugation batch had been transferred. The process was slow and there was some leakage at the exchange coupler connections that was resolved with the use of parafilm tape *. Once complete, another six centrifuge pots were filled with fruit puree and the process described above repeated.

Unfortunately some problems were encountered during the centrifugation of the fruit extracts that resulted in some delays to the process. Some of the centrifuge pots pulled a vacuum during centrifugation and became stuck in the rotor buckets. This was found to be due to an adequate seal on the pots due to the presence of residual fruit pulp on the lips of the centrifuge pots. Subsequently good care was taken to ensure a complete seal was achieved for each pot. As a further preventative measure, the temperature of the centrifuge was increased from 10°C to 20°C. Towards the end of processing it was discovered that the centrifuge pots used were only rated to 7000rpm (12227g). The speed was changed accordingly for the last centrifugation batch performed for the peach extract. The process was completed at 13:30, 8th June 2006.

* No recommendation has been made to address this problem as future bioprocess runs will be performed at a larger scale and will not require the use of exchange couplers.

Photo 4: Jane Talbot with a bag of Paw Paw Extract

19 bags of extract were produced from the fruit pulp. Stock lists were prepared for each extract prepared for the purpose of future stock reconciliations. Sample details, bag weights and % Recovery of extract from starting fruit pulp are recorded in the table below:

Table 3: Fruit Extracts Produced and % Recovery from Fruit Pulp

BAG NO.	ID/DESCRIPTION	NET WEIGHT (g)	TOTAL WEIGHT (g)	% RECOVERY OF EXTRACT FROM PULP*
1	Opal A/Paw Paw S	911.4g	7320.1g	74%
2	Opal A/Paw Paw S	912.7g		
3	Opal A/Paw Paw S	874g		
4	Opal A/Paw Paw S	908.3g		
5	Opal A/Paw Paw S	908.1g		
6	Opal A/Paw Paw S	912.7g		
7	Opal A/Paw Paw S	949.0g		
8	Opal A/Paw Paw S	943.9g		
9	Opal A NH/ Paw Paw NH	919.1g	1402.9g	61%
10	Opal A NHB/ Paw Paw NH	483.8g		
11	Opal A/ Paw Paw NHB	920.5g	1871.1g	77%
12	Opal A NHB/ Paw Paw NHB	950.6g		
13	Opal A H/	935.1g	1494.5g	63%

	Paw Paw H			
14	Opal A H/ Paw Paw H	559.4g		
15	Opal M/Peach S	927.4g	4131.8g	44%
16	Opal M/Peach S	935g		
17	Opal M/Peach S	974.5g		
18	Opal M/Peach S	924.3g		
19	Opal M/Peach S	370.6		

* % Recovery: Weight of Fruit Extract x 100
Weight of Fruit Pulp

Recovery of Paw Paw extract from pulp was significantly greater than recovery of peach extract from pulp. In particular, the standard Paw Paw process yielded a 74% recovery in contrast to the peach standard process which only yielded 44%. The reason for the yield variation between the paw paw and the peach process is surprising as there was no recollection of significant losses occurring during centrifugation, despite the problems encountered.

BLENDING

Blending of Paw Paw and Peach Extracts occurred on the 9th of June 2006 within the Seed Lots Laboratory (CJ38). The process was observed, in part, by Tom McArthur. This section of the manufacturing process was referred to as batch #6003.

Four bags of Paw Paw extract and one bag of Peach extract (batch #6002) were retrieved from 2-8°C storage for blending. Bags containing extracts were disinfected and placed into the BSC along with a balance, a peristaltic pump, tubing and exchange couplers. Opal001 and Opal002 blends were prepared by connecting one bag of Paw Paw extract with a bag of peach extract via an exchange coupler and then, using a peristaltic pump, transferring the required amount of extract from one bag to the other to achieve the desired ratio. The purpose of this closed-system was to protect material from potential contamination and to minimise the generation of aerosols. However a notable disadvantage was that it was difficult to achieve a high level of accuracy as minor movement of the bags and their associated tubing and connections was sufficient to cause fluctuations in weight measurements on the balance. During this process, and at previous steps in the manufacturing process, bags containing pulp/extract were weighed with tubing and connections intact. A bowl was used to contain the bag as the balance used was not large enough to hold the bag on its own (a larger balance would not fit in the BSC).

Blend Opal001 consisted of a 1:10 ratio of Paw Paw to Peach extract. Four bags of Opal001 were prepared, labelled Opal001 and Batch 6003/Bag No., and transferred to the Seed Lots fridge for 2 - 8°C storage.

Blend Opal002 consisted of a 1:1 ratio of Paw Paw to Peach extract. One bag of Opal002 was prepared, labelled Opal002 and Batch 6003/Bag No, and transferred to the Seed Lots fridge for 2 - 8°C storage.

Blend OpalA GFSE consisted of a 100:1 ratio of Paw Paw extract to Grape Fruit Seed Extract (GFSE). A syringe was used to add the GFSE to the tubing connected to the bag containing the paw paw extract. Unfortunately the viscous nature of the GFSE prevented its movement from the tubing in to the bag. To remedy this problem, an exchange coupler and tubing was connected to the bag and the peristaltic pump was operated in forward and reverse mode to dilute the GFSE with paw paw extract and allow for its transfer in to the bag. Unfortunately the small quantity of GFSE required for this volume of blend, and the blending process itself, resulted in the ratio being 20% greater than intended. One bag of OpalA GFSE was prepared, labelled OpalA GFSE and Batch 6003/1, and transferred to the Seed Lots fridge for 2-8°C storage. Stock lists were prepared for each blend prepared for the purpose of future stock reconciliations (refer to Appendix II).

Table 4: Blends Prepared from Fruit Extracts

BLEND ID	NET WEIGHT	DESIRED RATIO	ACTUAL RATIO
Opal001 6003/1	1242.2g	1:10	1.25:10
Opal001 6003/2	1002.6g	1:10	1.11:10
Opal001 6003/3	973.1g	1:10	1.11:10
Opal001 6003/4	989.4g	1:10	1.07:10
Opal002 6003/1	936g	1:1	1.01:1
OpalA GFSE 6003/1	881.4g	100:1	119:1

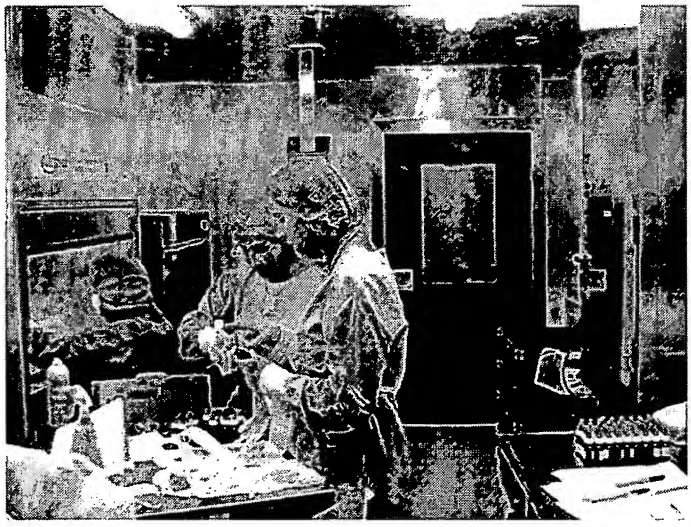
Photo 5: Transfer of blends to Seed Lots Fridge following completion of blending process.

DISPENSING

Dispensing of Paw Paw and Peach Extracts and Blends occurred on the 9th, 14th and 16th of June 2006 within the Seed Lots Laboratory (CJ38).

The dispensing apparatus was set up in the BSC. Set up involved connecting the filling needle to one end of the sterile tubing and attaching it to the filling needle stand. The other end of the tubing was aseptically connected to an exchange coupler which, in turn, was connected to one of the bags to be dispensed. The tubing was inserted into the dispensing pump which was positioned immediately adjacent to the BSC. The dispensing pump was calibrated for each Opal blend/extract to ensure product was dispensed in 10mL±1mL volumes. Aside from some wastage during initial calibration, the dispensing procedure performed as expected without any major incidents.

Photo 6: Dispensing and labelling of vials



Vials were labelled, placed in trays and then transferred to the Freezer Room (CJ10) for -80°C storage along with remaining bags of undispensed product. Stock lists were prepared for each blend/extract dispensed for the purpose of future stock reconciliations (refer to Appendix III).

Table 5: Blends and Extracts Dispensed

BLEND/EXTRACT ID DISPENSED	BLEND/ EXTRACT	DATE DISPENSED	NO. OF VIALS FILLED AND APPROVED	% REJECTED
Opal001 6003/2	Blend	09-June-06	90	6%
Opal001 6003/3	Blend	09-June-06	84	5%
Opal002 6003/1	Blend	09-June-06	93	1%
OpalA GFSE 6003/1	Blend	16-Jun-06	86	2%
Opal A	Extract	14-Jun-06	72	3%

Opal M	Extract	14-Jun-06	90	4%
Opal A NH	Extract	14-Jun-06	91	11%
Opal A NHB	Extract	16-Jun-06	93	2%
Opal A H	Extract	16-Jun-06	93	2%

Key: Opal001 - 10:1 Paw Paw to Peach Ratio (blend) Opal002 – 1:1 Paw Paw to Peach Ratio (blend)
Opal A – Paw Paw only (extract) Opal M – Peach only (extract)

The labels used on the vials were not ideal for freezing as it was originally stated that the product would be refrigerated.

QC TESTING

All QC testing was performed on product samples manufactured in the bioengineering run described in this report.

Bioburden Testing

It was initially intended that Opal products would be manufactured as non-sterile products and that a bioburden test would be required to quantify microbial content. Some initial testing has been performed on Opal001 and the Opal002 samples for the purpose of developing a bioburden test method.

When developing a bioburden test method it is necessary to assess and confirm whether the product that will be tested contains any substances that may inhibit the growth of any microorganisms that may be present. In the case of the Opal001 and Opal002, there are no substances present that are thought likely to cause any antimicrobial activity, however the alkaline nature of the product could have some inhibitory effect on microbial activity. Initial tests performed for the purpose of developing a bioburden method are described below:

Summary of testing

Tryptic Soy Agar (TSA) plates were streaked in duplicate with 10µL samples of Opal001 and Opal002 and incubated for 2 days at 37°C followed by 3 days at 23°C.

SAMPLE	PLATE 1	PLATE 2
Opal001	No growth	No growth
Opal002	No growth	No growth

As this testing was performed using only 10µL of sample, a decision was made to repeat the test using a larger sample spread over triplicate TSA plates. This testing was repeated using 1mL samples and the results were as follows:

SAMPLE	PLATE 1 – ANAEROBIC (37°C for 2 days)	PLATE 2 – AEROBIC (37°C for 2 days)
Opal001	No growth	1 CFU
Opal002	4 CFU	1 CFU

CFU – Colony Forming Units

In addition, Q-Gen performed sterility testing of these two products. 1mL of each product was inoculated into two types of growth support media: Thioglycollate (TGC), for the presence of anaerobic contaminants, and; Tryptic Soy Broth (TSB), for fungal and aerobic contaminants. TGC samples were incubated for 14 days at 37°C and the TSB for 14 days at 23°C. Final results of this testing are still pending, however results following 8 days incubation are as follows:

SAMPLE	TGC	TSB
Opal001	No growth	Possible growth
Opal002	Growth	Growth

These results support the second set of bioburden test results and indicate that both products contain some level of bioburden. The isolates observed in Opal002 have been sent for identification and results are still pending.

Antimicrobial activity validation

To verify that bioburden and sterility testing are not being affected by the presence of antimicrobial substances or by pH, some spike tests were performed to determine if microorganisms could be recovered. A microorganism standard solution, containing 10-100CFU *Staphylococcus aureus* per 100µL, was prepared for the following tests:

Partial sterility validation: 1mL of Opal001 and Opal002 were added to TSB. The TSB was then spiked with 100µL of *Staphylococcus aureus* standard and incubated for 3 days at 37°C to check for growth.

Partial bioburden validation: 100µL of Opal001 and Opal002 were each spread on to TSA plates. The product samples were allowed to dry on the plate. The plates were then spiked with 100µL of *Staphylococcus aureus* standard and incubated for 3 days at 37°C to check for growth.

SAMPLE	TSB + *S.aureus*	TSA PLATE + *S. aureus*
Opal001	Growth	Growth
Opal002	Growth	Growth

These results indicate that there is no significant anti-microbial effect demonstrated by the Opal001 and Opal002 product.

Bioassay

The bioassay developed by the Princess Alexandria Hospital, using human fibroblasts, is currently being trialled using Opal001 and Opal002. Results from this testing are still pending, however were not part of this contract.

HPLC

Unfortunately no HPLC results are available for inclusion in this report. The HPLC system has been in constant use for another project currently underway at Q-Gen and therefore has not been available for the analysis of Opal samples. The intention is to perform a comparison between filtered and unfiltered Opal001 and Opal002 samples for the purpose of determining whether a terminal filtration step can be added to the bioprocess schema. Results from this testing were not part of this contract.

CONCLUSIONS

The bioengineering run was completed successfully, albeit with some minor complications that fortunately were resolved without any apparent detrimental effect on product quality. The bioprocess schema used for this process is therefore considered acceptable and scaleable for phase I GMP clinical lot manufacture. However it is expected that the bioprocess may undergo some modification during further scale-up by a large scale contract manufacturer (for example, scale-up from tubing to hard-piping and the inclusion of continuous heating equipment).

RECOMMENDATIONS

It is recommended that an additional bioengineering run be performed prior to commencing process qualifications and clinical lot manufacture. This will facilitate the development of a more robust and reliable process and ensure a more seamless transition from technology development to GMP manufacture. It is proposed that the following suggestions be considered for implementation.

Develop and implement a more efficient and effective means of pureeing fruit. Possible suggestions include:

- Sourcing impeller blades that are able to puree and mix fruit efficiently while minimising the potential occurrence of "dead-zones" within the mixing tank. A current proposal is to model the design of impeller blades on those used in domestic food-processor. Two or more impeller blades may be required to minimise potential "dead-zones".

- Souring an industrial mincer to puree the fruit prior to its addition to the mixing tank, thereby negating the need for a pureeing impeller.

Additional bioengineering run to be performed at a larger scale. It is recommended that a minimum of 25kg of fruit puree be prepared in the mixing tank. Some of the heating difficulties encountered during the engineering run were attributed to an insufficient volume of pulp being directly subjected to heat from the tank jacket (the jacket does not extend to the base of the vessel).

Develop and implement a better method for heating fruit pulp. The heating method utilised in the bioengineering run, particularly the direct injection of steam in to the tank jacket, is not recommended for future runs as the likelihood for a temperature overshoot is significant. It is suggested that alternative means, such as the implementation of a thermocirculator, be considered to improve jacket heating efficiency and temperature control.

Install a viewing window, a light, or both, on the mixing tank. Such an addition to the tank would improve the process operator's ability to view the product during pureeing, heating and the addition of Sodium Bicarbonate.

Amend the bioprocess schema to include a second centrifugation step. Clarity of supernatant was significantly better following a second centrifugation and is likely to reduce potential problems from occurring if a final filtration step is implemented in to the manufacturing process.

Develop and implement a better system of weighing bags to ensure accurate in-process material weight measurements. As discussed previously in this report, concerns were raised during the blending process, in particular, during the preparation of Opal001 and Opal A GSFE, as these blends required the addition of small quantities of peach and GFSE, respectively. Variations in the tubing and/or exchange coupler connections, the positioning of bags on balances, and the movement of these items contributed to weight fluctuations which were felt to be significant contributors to inaccuracies and inconsistencies observed during the blending and weighing of extracts. A compromise may have to be sought between the desire to maintain closed-systems, as evident in this bioengineering run, and the need to have accurate weight measurements.

Bags should be pre-numbered with a unique identification number. This will avoid confusion when labelling multiple bags containing the same type of in-process product.

Develop a method for the accurate addition of Grape Fruit Seed Extract (GFSE). GFSE is highly viscous and is not easily transferred via peristaltic tubing. A suggestion is to reduce viscosity by diluting GFSE with a known quantity of the material it is intended to be blended with.

Final product labels are to be sourced and their suitability assessed. The adhesive on labels must be water-proof (or, at a minimum, water-resistant) and be effective at storage temperatures down to -80°C.

Include a terminal sterilisation step in the process to produce a sterile product. Since the bioengineering run was completed, Q-Gen have been advised by the TGA that products intended for treatment of open wounds is required to be sterile. As evident by the bioburden and sterility testing results for Opal001 and Opal002, the current method of preparation does not yield a sterile product. Therefore a terminal sterilisation method (such as filtration) needs to be developed. The potential effect on product efficacy of the chosen sterilisation method will need to be thoroughly assessed.

Further develop and validate a sterility test method. The small amount of work performed to develop a sterility test method was performed using a direct inoculation method. This method is limited in terms of product volume which can be tested at a single time. More development for a larger scale test method (possibly using membrane filtration) is required. In addition, testing was performed using only one standard microorganism (*S.aureus*). According to the TGA GMP Sterility Testing Guidelines, a further five microorganisms would need to be used.

For the purposes of product release, further assays are required to ensure purity, potency and quality. Q-Gen recommends HPLC for quantification and for determining purity and identity. This analysis will need to be combined with other methods, depending on the type of molecule the active compound is (for example, SDS-Page, Western Blotting. Amino Acid Analysis). Potency could potentially be a quantitative bioassay or an ELISA raised to the identified active compound. A stability trial should also be established so that the shelf life of the product can be determined in advance of clinical manufacture. It is recommended that Phoenix Eagle Company Pty Ltd also enquire as to the toxicological requirements for a topic open wound product.

APPENDICES

APPENDIX I

Draft Protocols:

OPAL BR-002 Preparation of Paw Paw and Peach Pulp (x2)

OPAL BR-003 Preparation of Fruit Extract (x1)

OPAL BR-004 Blending/Filling of Opal A, Opal M and Opal001, Opal002 (Part I & II)

APPENDIX II

Intermediate Product Stock Cards (Extracts & Blends):

Paw Paw S

Paw Paw NH

Paw Paw NHB

Paw Paw H

Peach S

Opal001

Opal002

Opal A GFSE

APPENDIX III

Final Product Stock Cards (Vials):

Opal001 6003/2

Opal001 6003/3

Opal002 6003/1

Opal M

Opal A

Paw Paw NH (Opal A NH)

Paw Paw NHB (Opal A NHB)

Paw Paw H (Opal A H)

Opal A GFSE

EXHIBIT #2 -19 pages

Case Reports

Participants Using

OPALA Filtrate and Cream

Prepared by: Dr Lyn Tozer
Medical Director
Datapharm Australia Pty Ltd

Date: 15 December 2009

SUMMARY

Participants at the Quadriplegic Centre, Perth^, agreed to the use of OPALA filtrate and cream to treat chronic pressure ulcers of the feet. These participants had spinal injuries (two quadriplegia, one paraplegia) and were interviewed, and the site of ulcers examined, by an independent medical practitioner*, on 7 May 2009. The results of treatment with OPALA filtrate and cream, compared to the previously used best practice treatment regimens, are especially remarkable given the expertise of the nursing staff of the Quadriplegic Centre in providing quality care of pressure ulcers in spinal injury patients, which was the one constant in all treatment regimens.

Participant QC14 received only four applications of OPALA treatment to ulcers on both heels when treatment with the filtrate ceased in response to apparent deterioration. The ulcers were exacerbated by additional pressure from the bed-end which occurred on the first night of OPALA treatment. Application of OPALA cream to the skin surrounding ulcers on both heels and to the legs continued. While the photographic record confirms that the centre of the left heel ulcer was deeper with necrotic eschar at the base, it is notable that the necrotic tissue and slough that had been adherent to the margins of this ulcer was no longer present and the condition of the surrounding skin had improved.

Participant QC13, a long term quadriplegic with insulin-dependent diabetes, had an ulcer on his right foot which had been present for five months at the time treatment with OPALA was commenced. At this time the ulcer was deteriorating, with slough and necrotic tissue present, and was being treated with best practice dressings and repeated courses of antibiotics. After initiation of treatment with OPALA filtrate and cream the ulcer remained clean without the need for further antibiotics. Despite repeated trauma to the ulcer, involving bumping the area during wheelchair outings, the ulcer remained clean and continued to decrease in size between these incidents. At examination, five months after commencement of the OPALA treatment, there was no necrotic tissue, slough or exudate and the ulcer was almost healed.

Participant QC15, a relatively recent paraplegic, with a history of eczema, dermatitis and long-term steroid use, had a chronic pressure ulcer on her left foot which had been present for nearly seven months at the time treatment with OPALA was commenced. A number of different best practice treatment regimens had been tried and antibiotics were prescribed almost continuously during this period. After starting treatment with OPALA filtrate and cream only one further course of antibiotics was prescribed and the ulcer was completely healed in eight weeks. At examination the ulcer had been healed for six weeks and there was no sign of recurrence.

Both QC13 and QC15 received treatment with OPALA products for several weeks, allowing the ulcers to become clean and debrided of surrounding dead tissue and slough. It appears that this initial debriding action of OPALA lead to progressive improvement in the appearance and size of the ulcers without the need for other physical or chemical debridement or repeated courses of antibiotics which had been the recurring pattern of prior treatment regimens. In the case of QC15 healing was complete in eight weeks and was sustained, with the skin over the site of the healed ulcer appearing healthy and normal six weeks after closure.

^ The Quadriplegic Centre, Perth, is a 100 bed hospital providing specialist management of people with the most severe high spinal cord injuries and spinal cord disease. The QC employs specialist Clinical Nurses and has established itself as a centre of excellence in the area of prevention and management of pressure areas.

* Dr Lynette M R Tozer, MBBS, MHA, Medical Director, Datapharm Australia Pty Ltd. A GP for several years, the author has 15 years experience in the pharmaceutical industry with responsibilities including clinical study management, pharmacovigilance and medical writing.

Case Report: Participant #QC13

Author: Dr Lynette M R Tozer, MBBS, MHA, Medical Director, Datapharm Australia Pty Ltd. A GP for several years, the author has 15 years experience in the pharmaceutical industry with responsibilities including clinical study management, pharmacovigilance and medical writing.

Date of Review: 7 May 2009

Synopsis

This 58 year old male has been a C5/6 quadriplegic since 1980 when he was involved in a motor vehicle accident. He is an insulin-dependent diabetic who is mobile in an electric wheelchair and who has a history of ulceration and injury to his lower limbs over a number of years. The ulceration on the soles of his feet has been present since early 2006 and has failed to heal completely despite treatment with best practice dressings, debridement and with multiple courses of antibiotics. Treatment with OPALA filtrate and cream was commenced on 10 December 2008 after which the participant required no further antibiotics. The partial resolution of the wound followed a turbulent course due largely to the participant's non-compliance with medical advice for bed rest and lack of appropriate care in manoeuvring his wheelchair. On 7 May 2009 the ulcer was clean and dry and measured 2 mm in diameter. Details included in this case history were obtained from medical notes held at the Quadriplegic Centre (QC) and Royal Perth Hospital (RPH), discussions with nursing staff and an interview with the participant, including examination of the ulcer.

Participant Profile

The participant is a Caucasian male born in 1950. He was aged 30 years when he was admitted to RPH after running his moped into a stationary motor vehicle. He was wearing a helmet, however, the force of his impact smashed the rear window of the vehicle and he was catapulted over its roof and onto the roadway. He was immediately aware of extreme pain in his neck and the inability to move his limbs. Spinal injury occurred at the C5 level and he has subsequently regained some arm movement and with that, the ability to operate an electric wheelchair.

Background and Medical History

The participant has a long history of insulin dependent diabetes (1990) which was initially controlled by diet but which now requires insulin three times daily. He reported having eczema as a boy and also developing a severe generalised rash, thought to be due to inhaling steam (and chemicals) used to recondition metal containers in his workplace prior to his spinal injury. He also has hypertension and gastrooesophageal reflux disease and is allergic to penicillin, norfloxacin and Fixomull® as well as to certain foods (stone fruit, tomato, crab).

In 1995 the participant had an inflammatory lesion on his left shin with blisters and discolouration which was treated with gelonet dressings. Again in 2004 swabs of similar lesions on both shins grew non-MRSA. He stated that he first suffered from lesions on his shins over 20 years ago following severe sunburn.

The participant has a long history of recurrent urinary tract infections and urinary calculi and a suprapubic catheter was inserted in 2005. Also in 2005 he was diagnosed with neurogenic bladder and had surgical repair to bilateral hydrocoeles. In 2006 he was diagnosed with gastric dilatation and in January 2009 blood test results confirmed diabetic renal damage.

Since his spinal injury the participant has had a history of repeated injuries to his lower limbs, particularly his feet and toes. A pressure ulcer first appeared on the lateral side of the sole of his right foot in 1982, caused by poorly fitted shoes. This initial ulcer was present for several months and the skin in this area has remained fragile, the ulcer reforming at this site many times during the intervening years. In addition, in January 2006 an ulcer was reported on the sole of his left foot adjacent to the 2nd toe with surrounding cellulitis, requiring antibiotic treatment (erythromycin) and daily dressings for several weeks. In April 2006 he was again found to have excoriations on his shins which were treated with Fixomull® and betadine and which persisted for six months.

Also in April 2006 an ulcer was found on the ball of his right foot below the 5th toe which persisted for several months. In October 2006 this ulcer was approximately 1 cm in diameter and covered by hard crust. Daily dressings were applied, after cleaning with normal saline and application of Intrasite gel, however, after five days the ulcer was reported to be sloughy with moderate ooze, slight odour and surrounding redness. The participant was treated with cephalexin (1g bd) and the ulcer dressed initially with Algisite and later with Purilon, however, the wound continued to deteriorate and bed rest was advised. By 11 November the size of the lesion was 1.5 cm in diameter. Treatment consisted of cleaning with normal saline followed by covering the ulcer with Allevyn. This wound care plan continued daily for several weeks.

In mid-January 2007 the ulcer was noted to be partially covered by slough and a further 12 days of cephalexin was prescribed. By the end of the month the ulcer was clean with a small area of epithelialisation around the edges and the wound care plan continued. On 28 March 2007 the participant was once again reported to have a lesion on his left foot and in May 2007 the right foot wound recurred and was infected with slough and redness of the surrounding skin. Dressings were applied according to best practice and after a few weeks the pressure areas on both feet showed signs of healing with epithelialisation and absence of both exudate and surrounding redness. The right foot ulcer was reported as being healed on 15 June 2007.

The participant continued to have exacerbations of the lesions on his shins from July until November 2007. The skin on his shins and below the right little toe was reported to be fragile, however, there were no reports of ulceration again until July 2008.

History of the Present Problem

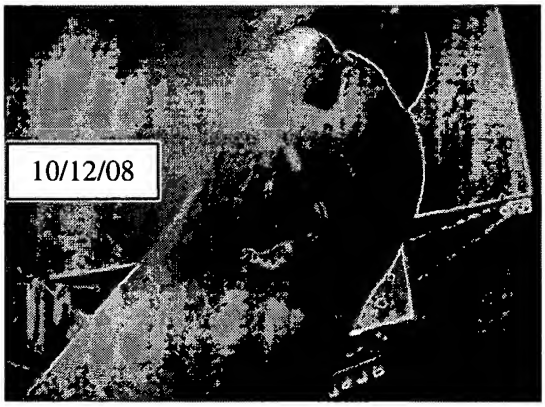
On 7 July 2008 the participant spun in his electric wheelchair and bumped his right foot against the side of the chair causing a break in the skin on the outer aspect of the right foot below the small toe (4-5th metatarsal) which was inflamed with possible early cellulitis. A dressing was applied and he was advised to rest in bed. When the wound was reviewed after two days, cellulitis was definitely present around the wound which was discharging pus. Iodosorb ointment was applied and he was prescribed cephalexin (1g bd) and bed rest. He remained in bed the following day when his right shin was observed to be red and hot and his right foot swollen with oedema. Antibiotic therapy was changed to ciprofloxacin (500 mg tds), the wound was debrided and Inadine applied. By 13 July the cellulitis was receding but there was no apparent healing of the ulcer.

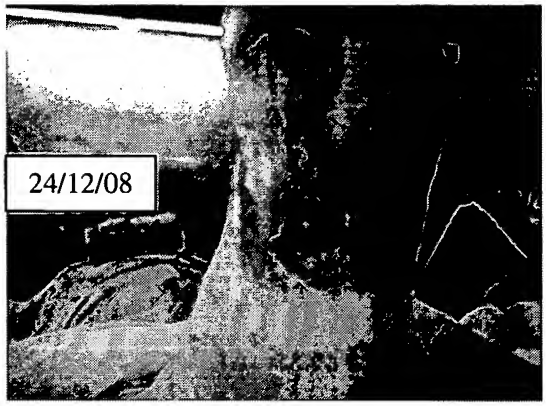
On 22 July the ulcer appeared smaller (2.5 cm diameter) with a small amount of exudate, slough and granulation tissue. It was cleaned and covered with PolyMem Silver® and three days later antibiotic therapy ceased. From early August Mesalt dressings were applied to the ulcer which continued to improve during the next few weeks. On 13 September the ulcer was continuing to heal and PolyMem dressing was applied, however, on the following day the wound had deteriorated and ciprofloxacin (500 mg bd) was commenced. It was postulated at this time that the foot plate of the participant's electric wheelchair may have contributed to the ulcer's failure to heal and adjustments were made.

By the end of September 2008 antibiotic therapy had ceased, the wound was once again clean but with a moderate amount of clear exudate. A new dressing plan was established for the right foot which was to be left for seven days between reviews but the initial application fell off during the first night and was reapplied. The ulcer failed to show signs of healing and on 22 October another course of ciprofloxacin (500 mg bd) was commenced. Continued deterioration followed and on 6 November the presence of necrotic tissue was noted around the wound. On 14 November the participant was advised to rest in bed however he largely disregarded this advice and the ulcer continued to deteriorate.

Treatment with OPALA Products

Daily application of OPALA filtrate and cream to the ulcer in his right foot below the small toe (4-5 metatarsal) was commenced on 10 December 2008. After five days, while the ulcer appeared to be the same size, it was clean. Three days later there was bleeding from the base of the ulcer possibly from the formation of granulation tissue. Gradual improvement followed and on 24 December the ulcer was noted to be clean with some granulation at the edges.

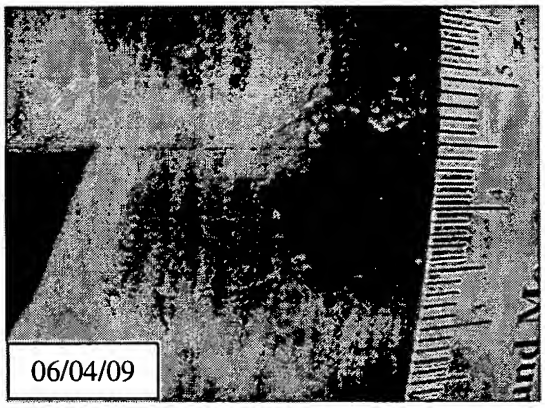




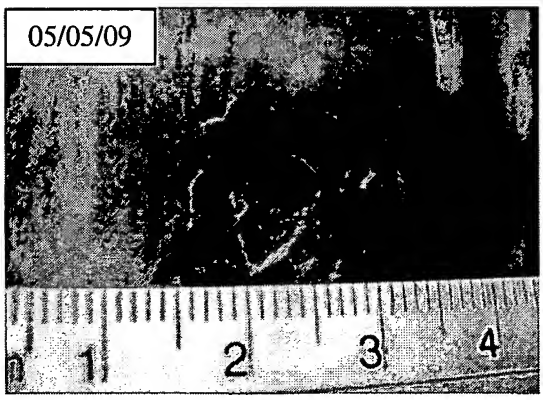
The right foot ulcer remained clean and had reduced in size by 15 January 2009, however, on 3 February once again there was deterioration which appeared to have been due to trauma to the area. The participant denied that this was the case.

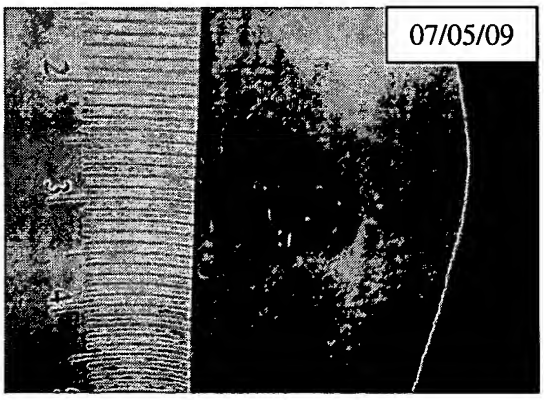
Dressings with OPALA continued, the participant was more compliant with bed rest and by 13 February the ulcer was once again noted to be improving. It was clean and reduced in size to 0.5 cm diameter. Six days later (19 February) the participant knocked his right foot while in the shower, as it fell off the foot plate of his wheelchair. Subsequently a strap was provided for use in the shower. The participant was involved in several incidents involving both the speed and turning action of his wheelchair so a speed-limiter was installed to his chair to attempt to reduce these incidents and limit the damage he inevitably caused to himself. On 13 March 2009 the ulcer on his right foot was notably improved, both cleaner and smaller. The improvement continued and on 23 March the area required a smaller dressing wick.

From 2 to 7 April the wound had decreased in size and depth (less than 1 cm) and was noted to be much improved. However, the following day it was soaked with fresh blood and the skin around the wound was severely bruised with petechial haemorrhage over the dorsum of the foot. Despite evidence to the contrary, the participant once again denied the occurrence of trauma to the area. Only the OPALA filtrate was applied to the broken skin, the area was washed with aqueous cream to remove the OPALA cream and bed rest was advised.



By 15 April the ulcer on the participant's right foot showed significant improvement and most of the bruising was gone. There was continued improvement and the participant abided by medical advice to rest in bed to assist the healing process. On 5 May 2009 the ulcer was clean and had reduced in size to 0.5 cm diameter. Two days later the ulcer was less than 0.2 cm in diameter.





Outcome

At the time of reporting the ulcer on the participant's right foot, which had been present continually for five months prior to initiation of treatment with OPALA products, was clean and almost healed. Pressure ulcers on both feet had been intermittently present over many years and the involved areas of skin had become fragile and easily damaged. Despite his longstanding quadriplegia this participant made every attempt to be mobile and independent and resisted extended periods of bed rest. The healing process after commencement of OPALA was delayed by several episodes of trauma to the affected area, however, the wound remained clean and the participant did not require antibiotics during this time. Healing has allowed the participant to remain active and to undertake his limited work responsibilities at the QC.

Discussion and Conclusions

This participant has been a quadriplegic for 29 years and lives at the QC where he is employed to oversee staff and patient parking and mobility is of major importance to his sense of wellbeing. The chronic ulcers on his feet were initially due to pressure of poorly fitted shoes which left the skin fragile and easily damaged. He has a multitude of medical conditions in addition to and as a result of his spinal injury, including insulin dependent diabetes, which impact on the incidence of recurrence and infection of his foot ulcers as well as the tendency for them to heal slowly. Until treatment commenced with OPALA filtrate and cream various wound care plans had been in operation which included best practice cleaning techniques and dressings, supported by antibiotic therapy and debridement when required. The ulcer on the participant's right foot below the 5th toe was present for five months prior to initiation of treatment with OPALA filtrate and cream. The participant was persistently non-compliant with bed rest and often compromised the healing of his foot ulcers which were certainly exacerbated by the injuries sustained while manoeuvring his wheelchair.

This pattern of non-compliance continued after OPALA treatment had begun, however, it is notable that despite the repeated injuries he sustained after this time which caused the ulcer to break down and enlarge, the ulcer remained clean and no further antibiotics were prescribed. This had not been the experience prior to institution of OPALA treatment and it is possible that the success of the OPALA products in cleaning away the slough and necrotic tissue due to its apparent debriding action, also kept the ulcer clear of infection.. The clean, debrided ulcer was then allowed the opportunity to heal.

When the ulcer was examined on 7 May 2009 there was no sign of necrotic tissue, slough or exudate. It was noted by the attending nursing staff that it was obviously smaller than it had been two days previously. There was no evidence of recent trauma to the site or surrounding area indicating that the restrictions imposed on the speed and manoeuvrability of the participant's wheelchair may have had the desired effect.

Case Report: Participant #QC14

Author: Dr Lynette M R Tozer, MBBS, MHA, Medical Director, Datapharm Australia Pty Ltd. A GP for several years, the author has 15 years experience in the pharmaceutical industry with responsibilities including clinical study management, pharmacovigilance and medical writing.

Date of Review: 7 May 2009

Synopsis

This 65 year old male has been a quadriplegic for 16 months following fracture of the odontoid process in a surfing accident. He is intubated and ventilated and only mobile in an electric wheelchair with the assistance of staff. He developed ulcers on the heels of both feet following intensive physiotherapy involving an electric bicycle. These ulcers were exacerbated by repeated malposition in bed and had been present for over two months when treatment with OPALA filtrate and cream commenced on 18 December 2008. Treatment with the filtrate was discontinued on 22 December as the ulcers appeared to have deteriorated, however, the application of the cream to the surrounding skin continued. Photographs of the left heel show that the centre of the ulcer was deeper with necrotic eschar, but without the slough and necrotic tissue at the margins which had been present at the start of treatment. This latter may be due to the debriding action of OPALA. Improvement in the condition of the surrounding skin was also noted. Treatment of the ulcers with standard best practice therapies continued after this time. Details included in this case history were obtained from medical notes held at the Royal Perth Hospital (RPH) Spinal Unit, discussions with nursing staff and an interview with the participant, including examination of the ulcers.

Participant Profile

The participant is a Caucasian male, born in 1943. He was aged 64 years when he was involved in a surfing accident in 2007. At this time he sustained a Type 2 oblique fracture through the body of the odontoid process with mild retropulsion of the superior fragment. It was reported that the participant had jumped into a wave and was found lying face down in the water and dragged out by his family. CPR was performed by a bystander for 15 minutes prior to the arrival of paramedical assistance. He was taken by ambulance to Joondalup Hospital where he was intubated and ventilated prior to transfer to RPH. MRI on admission confirmed the nature of the spinal injury and showed cord oedema extending from the medulla to the level of C4 in addition to some cord haemorrhage. While in ICU, the participant developed aspiration pneumonia which was treated with meropenem trihydrate and ciprofloxacin. After his condition was stabilised he was transferred to RPH Spinal Unit.

Background and Medical History

The participant's medical history includes cervical arthritis requiring C4-5 and C5-6 discectomy with spinal fusion in 1988. In 1997 he had the procedure repeated at the level of C6-7. He is allergic to sulphonamides. Following his spinal injury his main medical concerns were repeated episodes of sepsis (pneumonia, recurrent UTIs) and persistent hypotension. His medication at the time this history was recorded included:

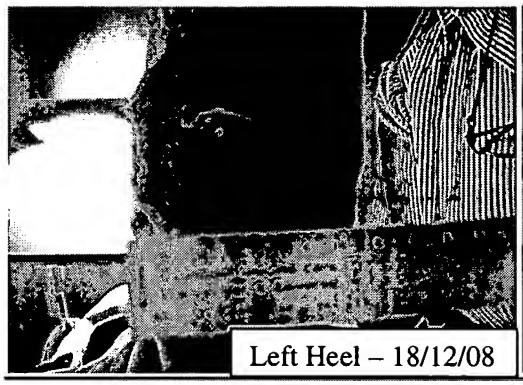
Medication	Dose/route	Medication	Dose/route
amitriptyline	75mg nocte	fludrocortisone	150mcg mane
bromhexidine	started 9/10/2008	ibuprofen	400mg tds prn
simethicone	after meals	Movicol	nocte
docusate and senna	bd	Sofradex	tds
domperidone	10mg bd		

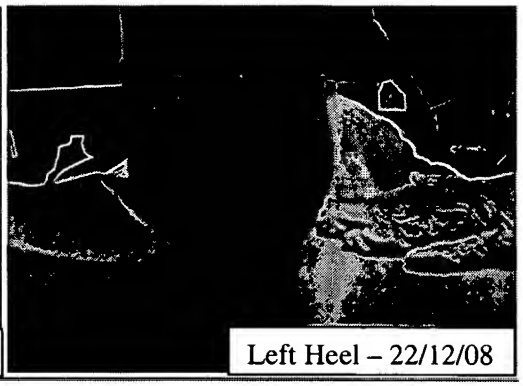
History of the Present Problem

On 3 October 2008 the participant commenced intensive physiotherapy which involved exercising on an electric bicycle into which he was strapped. On return to his bed staff noted that skin was missing from the heel of his left foot and that both heels were abraded. A wound care plan was prepared which provided for daily cleaning with normal saline, application of Intrasite to a blacked area in the ulcer and Jelonet to the remaining area of both heel ulcers. The entire area of both wounds was covered with Biatain and the heels covered with Allevyn and foam bootees for protection. At this time the ulcers were superficial, measuring 3 cm in diameter without exudate, slough or necrosis present and the surrounding areas of skin were pink. After six days Bactigras was substituted for Jelonet. The ulcers deteriorated and from 14 October the wound care plan was changed to include application of Iodosorb to the area prior to covering with Biatain and securing with microtape.

Treatment with OPAL Products

On 18 December 2008 the left heel was oozing through the dressing. Daily application of OPALA filtrate and cream to both heel ulcers was commenced on this date. The following day the participant was found to have slipped down the bed and his feet were pressing on the bed end, exacerbating the pressure areas on both heels. At this time he was repositioned, the heel wounds were reviewed and OPALA cream was applied to both legs When the wounds were reviewed on 22 December the wounds on both heels appeared to have deteriorated. The ulcers were reported to be larger and deeper and there was a large necrotic eschar at the base of the left heel ulcer. It was decided to discontinue treatment with the OPALA filtrate but to continue application of OPALA cream to the skin surrounding the ulcers and to the legs. Standard wound care was resumed including debridement of 1 cm of necrotic tissue from the right heel on 27 December.


Left Heel – 18/12/08


Left Heel – 22/12/08

Outcome

The use of OPALA filtrate was discontinued after four days, however, use of the cream around both ulcers continued. The ulcers on both heels were then managed using best practice wound care including dressings, antibiotics and debridement. A swab of the left foot ulcer on 19 January 2009 grew *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the participant was treated with antibiotics. Standard care was continued and at the time of reporting (7 May 2009) the wounds on both heels were still being dressed daily and healing had continued slowly.

Discussion and Conclusions

This participant suffered pressure ulcers on his heels as a result of vigorous physiotherapy. The ulcers had been present for over two months prior to commencement of daily treatment with OPALA filtrate and cream. The filtrate was applied only four times before being discontinued while application of the cream to skin surrounding both ulcers and to the legs continued. The photographs of the right heel could not be compared, however, those of the left heel above show that while the ulcer appeared larger on 22 December, with necrotic eschar at the base, there is a marked absence of the slough and necrotic tissue at the margins of the ulcer which had been present in the earlier photograph. This may be attributable to the debriding action of the OPALA products. It is also notable that the condition of the skin surrounding this ulcer had improved, appearing healthier in the later photograph.

Case Report: Participant #QC15

Author: Dr Lynette M R Tozer, MBBS, MHA, Medical Director, Datapharm Australia Pty Ltd. A GP for several years, the author has 15 years experience in the pharmaceutical industry with responsibilities including clinical study management, pharmacovigilance and medical writing.

Date of Review: 7 May 2009

Synopsis

This 49 year old female participant has been a T6 paraplegic for 15 months and is mobile in an electric wheelchair. She developed an ulcer on the sole of left foot due to pressure on the bed-end and the footplate of the wheelchair. The ulcer failed to heal with best practice therapies over a period of six months. Treatment with OPALA filtrate and cream was commenced on 20 January 2009 and the wound was completely resolved on 24 March after eight weeks of treatment. The ulcer showed no sign of recurrence several weeks later and the healed skin appeared healthy. Details included in this case history were obtained from medical notes held at the Quadriplegic Centre (QC), Royal Perth Hospital (RPH), Sir Charles Gairdner Hospital (SCGH), discussions with nursing staff and an interview with the participant including examination of both feet and hands.

Participant Profile

The participant is a Caucasian female, born in 1960. She was admitted to SCGH on 5 February 2008 for treatment of an infective exacerbation of longstanding severe asthma. Her condition rapidly deteriorated and she required BiPap. On 17 February 2008 the participant suffered tonic clonic seizures secondary to serotonin syndrome thought to be due to a combination of citalopram and other pain medication possibly tramadol hydrochloride or TCA. Due to long term high-dose steroid use for asthma the participant was known to have severe osteoporosis and the seizures caused retropulsion of the T6 vertebral body resulting in paraplegia. She was initially intubated, ventilated and stabilised as an in-patient at SCGH and was transferred to the RPH spinal unit on 24 April 2008 for continuation of treatment. On 28 July 2008 she went to the QC for rehabilitation and preparation for community placement.

Background and Medical History

The participant's past medical history includes asthma (lifetime), depression (10 years), hypertension, migraine and primary pulmonary hypertension. She stated that her past history includes severe eczema and dermatitis which had resolved prior to her spinal injury. The participant has cushingoid syndrome and osteoporosis secondary to long term (10 years) high dose steroid use for asthma. Allergies include aspirin, NSAIDs and propranolol, which caused leg swelling when used to treat migraine. She is obese and has been a smoker. The pain caused by osteoporosis had already impacted on her ability to care for her intellectually disabled adult son prior to her spinal injury.

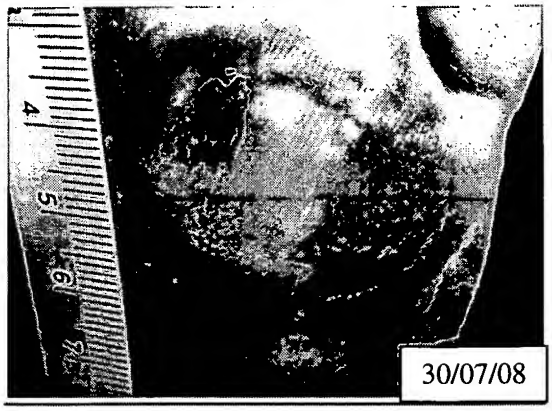
In January 2008 she received a bisphosphonate infusion for osteoporosis for which she was also using calcitriol and calcium carbonate. Prior to her spinal injury she had also received methotrexate for asthma and risperidone for paranoia. During the admission to SCGH due to the exacerbation of asthma and initial management of paraplegia the participant's concomitant medication included the following:

Medication	Dose/route	Medication	Dose/route
buprenorphine	600 mcg nocte	sodium valproate	200 mg bd
buprenorphine	Patch 40 mg/week	oxybutynin	2.5 mg bd
calcitriol	0.25 mg/day	Panadeine forte	2 qid
calcium carbonate	600 mg bd	predniosolone	7.5 mg daily
coloxyl+senna	2tabs daily	pregabalin	300 mg bd
diazepam	5 mg tds	quetiapine	125 mg nocte
esomeprazole	40 mg OD	salbutamol nebules	5 mg qid
frusemide	20 mg BD	Seretide 250/25	2 puffs bd
glycerine supps	Twice weekly	tramadol	50-100 tds prn
montelukast sodium	10mg nocte	venlafaxine	75 mg mane

Following the spinal injury the participant was given a trial of baclofen to control muscular spasms. The drowsiness and confusion, with hypotension, which she experienced at this time was possibly related to this medication and it was discontinued. During the admission to SCGH, hypotension due to adrenal insufficiency, secondary to long term steroid use, was managed with intravenous hydrocortisone. She developed persistent urinary tract infections due to *Klebsiella spp* and was treated with multiple courses of oral antibiotics followed by insertion of a suprapubic catheter. On 15 February 2008 the participant had an echocardiogram which demonstrated moderate pulmonary hypertension with mean pulmonary arterial pressure (PAP) of 30mmHg and right atrial pressure (RAP) of 5mmHg.

History of the Present Problem

On 17 July 2008 it was noted that the participant had increasing pedal oedema, which was worse when her legs were dependent. This was treated with diuretics after a Doppler examination showed no evidence of DVT. On 20 July a lesion on the sole of the participant's left foot was noted for the first time. The wound care plan at this time included daily cleaning of the serous exudate with normal saline, wiping with an alcohol swipe and covering with adhesive dressing. There was also concern for an area on the sole of the right foot which was treated using an alcohol swipe only. On 21 July the left foot lesion was dressed using conformable Intrasite and covered. The following day a swab of the ulcer grew *Staphylococcus aureus.* A few days later it was noted that the participant's feet were pressing hard up against the bed end, exacerbating the lesions on her feet and during the dressing procedure on 28 July, grazes were observed on both feet above the heels.



On 30 July the ulcer on the left foot was measured at 3x4 cm and inadine and Biatain were applied and covered with Fixomull®. The right heel continued to receive treatment with the alcohol swipe only.

By 4 August 2008 the left foot lesion was an open, discharging wound from which a swab was taken, however, no report was issued. It was observed at this time that there was also discolouration of the right foot wound.

After a week some improvement of the left foot ulcer was observed and elevation of the participant's feet was prescribed. Review of the left foot on 18 August showed slough in the centre of the ulcer but no erythema was present. The right foot wound was dry and healing. On 20 August the left foot ulcer had improved, and the same wound care plan was continued. The participant was advised to sit in her electric wheelchair for a maximum of four hours daily. Five days later the left foot wound was noted to be healing well. However, on 31 August the wound was observed to have increased in size and the participant was advised to stay in bed for 24 hours to aid the healing process.

On 1 September the wound on the left foot was treated according to the wound care plan and moisturiser was applied to other areas of both feet. Two days later the treatment plan was focused on elevation of the feet and the following day it was noted that the wound appeared to be slowly healing.

Healing continued for over a week at the end of which the ulcer on the left foot was nearly healed and required only protective dressings.

On 4 October 2008 the participant suffered acute extreme dyspnoea becoming cyanotic and drowsy with altered responsiveness and was admitted to SCGH for six days. Medication changes during this hospital admission included changes to doses of enoxaparin sodium, slow K, diazepam, paracetamol, buprenorphine and prednisolone. Hospital discharge notes recommended continued bed rest and tapering of her steroid dose. On her return to the QC she was noted to have pressure sores on her buttocks and a small necrotic area on the sole of her left foot which was treated according to the wound care plan (cleaning with normal saline, Intrasite conformable and dry dressing). On 21 October the ulcer on her foot was observed to be healing slowly.

On 25 October 2008 the participant was drowsy and febrile (38.9°C) and sent by ambulance to the RPH emergency department. She was admitted to RPH with temperature of 38.3°C, blood pressure 127/75 and with bilateral expiratory wheeze and bibasal crepitations on chest auscultation. ECG revealed right bundle branch block which may have been old and haematology examination showed haemoglobin 128 g/L, WCC noted to be normal, CRP 130 mg/L, GGT 86 IU/L and pCO2 of 49 mm Hg. Chest X-ray showed that the lung fields were clear with no evidence of consolidation. She was treated with hydrocortisone and vancomycin, her usual medications were continued in addition to ipratropium bromide nebules four times daily and salbutamol nebules increased to six times daily. Prednisolone was increased to 50 mg daily initially followed by graduated reduction to her usual dose. She was treated with ticarcillin sodium/potassium clavulanate for three days intravenously before being switched to oral amoxicillin trihydrate/potassium clavulanate (500 mg bd) and she commenced nightly temazepam (10 mg) as required. Since the previous admission her asthma medication had been changed to fluticasone 125/salmeterol 25 (2 puffs bd) and she had also been receiving metoclopramide (10 mg tds prn) for nausea and potassium chloride (2 tablets bd).

On 29 October 2008 the participant was returned to the QC still on amoxicillin trihydrate/potassium clavulanate (ceased 1 November) and temazepam plus her other regular medications. She stated that the wound dressings on her feet had not been attended during her hospital admission and staff notes indicate that the wound on the left foot had become necrotic and sloughy. From 6 to 13 November the wound was dressed daily with Aquacell silver, telfa and Fixomull®.

Swabs of ulcers on both feet on 17 November grew abundant *Staphylococcus aureus* and amoxicillin trihydrate/potassium clavulanate (500 mg bd) was commenced. The left foot wound was observed to be deep and sloughy. Other changes to medication at this time included oxycodone hydrochloride for pain (10-20 q4hrly prn from 21 to 24 November, reducing to 5-10 qid prn from 25 November to 30 December 2008) and by 11 December 2008 the participant's prednisolone dose had been reduced to 2.5 mg daily.

Cephalexin (1g bd) was commenced on 13 December 2008 (ceased 23 December) and the following day the left foot wound was observed to be getting smaller and there was no longer any discharge. On 22 December it was once again noted that the wound was not healing as well as it had been previously. Two days later the wound was deep with a bad odour and

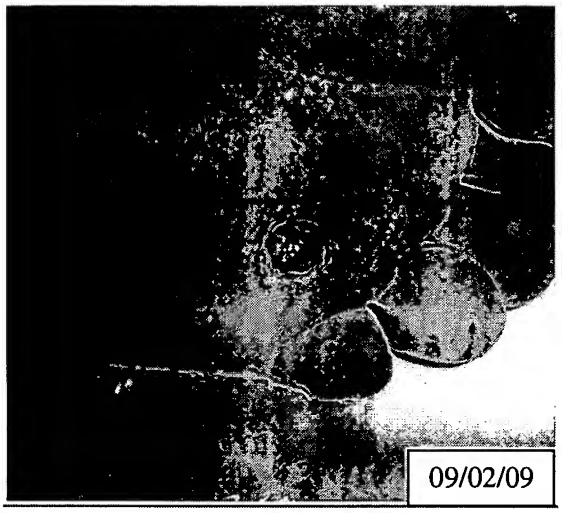
surrounding dead tissue. Iodosorb was applied, antibiotics commenced (diflucloxacillin 500 mg 6 hrly ceased 12 January 2009) and chemical debridement ordered. Later the same day Intrasite and Biatain were applied to the wound. Two days later when the dressing was changed, the wound was measured as 4 cm diameter and 3 cm in depth.

On 1 January 2009, though antibiotics had been continued, the wound was noted to be sloughy and was not improving. After five days there was some evidence of slow wound healing and it was cleaner than before. Three days later the wound was clean with no obvious granulation and was bleeding at the edges. By 12 January the wound was clean but still deep and two small blisters were also noted on the left sole. At this time the participant also complained of dry palms. The participant's sodium valproate dose had been increased at this time which was thought to be related to the dry skin.

Treatment with OPALA Products

On 20 January 2009 due to the depth and chronic nature of the ulcer an X-ray was taken of the left foot which excluded the presence of osteomyelitis. Thereafter, treatment with OPALA cream and filtrate was commenced. The size of the ulcer at this time was recorded as 1.5x1.0 cm with a depth of 1.3 cm.

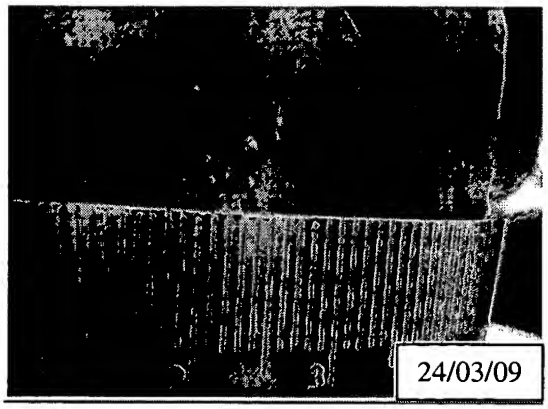
On 23 January changes were requested to the foot support in the participant's electric wheelchair as it was considered that this was contributing to the recurrent exacerbations of the foot ulcers particularly on the left foot. During January the participant was motivated to commence both a weight loss program and a smoking cessation program. When the ulcer was dressed on 31 January it was measured as 1.3 x 1.0 cm with depth of 1.0 cm.



On 2 February the left foot ulcer was observed once again to be improving. However, on the following day flucloxacillin (500 mg q6h) was started due to infection in the wounds in both feet and it was reported that the participant would not stay in bed as advised. Continued healing was noted on 7 February and again on 9 February. On 11 February the ulcer was measured as 1.0 x 1.0 cm, with depth of 0.4 cm. On 15 February healing of the left foot was noted to be slow and blisters were present on the right foot.

Healing of the foot ulceration continued and it was noted on 26 February that the wound on the left foot was healing well. On 9 March 2009 the left foot ulcer was getting smaller and the blisters on the right foot were dry. In addition to the OPALA products the participant was applying terbinafine cream to the palms of her hands and it was also applied to the right foot due to a clinical diagnosis of a fungal skin infection. She was seen by a dermatologist but no scrapings were taken to confirm the diagnosis.

On 15 March 2009 the ulcer on the left foot had almost healed and was noted by nursing staff to be the size of a pinhead. The following day the ulcer on the left foot was nearly healed and the right foot had only dry scabs. Application of terbinafine cream was continued to both hands and the right foot.



The left foot ulcer continued to heal well and was noted to be fully resolved on 24 March 2009. At this time application of the OPALA filtrate was discontinued and only the OPALA cream was applied to the left foot especially around the margins of the scar at the ulcer site.

The participant's concomitant medication during March 2009 included the following:

Medication	Dose/route	Medication	Dose/route
calcium carbonate	600 mg bd	oxybutynin	2.5 mg bd
diazepam	5 mg tds	Panadeine forte	2 qid
esomeprazole	40 mg OD	predniosolone	7.5 mg daily
potassium chloride	2 tabs bd	pregabalin	300 mg bd
metoclopramide	10 mg tds prn	quetiapine	125 mg nocte
montelukast sodium	10mg nocte	salbutamol nebules	5 mg qid
sodium valproate	200 mg bd	venlafaxine	75 mg mane
oxycodone	5 mg bd to tds prn		

Staff continued to apply the OPALA cream to both feet and hands from 30 March to treat a possible fungal infection which had resolved at the time of examination on 15 April 2009. On 22 April 2009 the participant was transferred from the QC back to the RPH spinal unit to await placement.

Examination of her skin on 23 April revealed that her palms were dry and indurated but no active fungal infection was clinically present. The left foot ulcer at the base of 4th metatarsal remained healed and there were no lesions present on the right foot. Changes to her concomitant medication at this time included salbutamol nebules to be used as required via an MDI, methyl salicylate cream to treat generalised dry skin and potassium chloride dose was increased (2 tabs tds).

Outcome

The healing of the pressure ulcers on her feet has significantly impacted the speed of physical rehabilitation and general well being of this participant. Recent acquisition of a new powered wheelchair further improved her comfort, pressure relief and mobility. Commencement of a self-motivated weight loss program from early 2009 has resulted in weight reduction of 11 kg at the date of reporting and she continues the smoking cessation program. She was attending occupational therapy and awaiting community placement which was complicated by a difficult social situation.

On 7 May 2009 the left foot ulcer remained healed with no sign of further breakdown of skin integrity and the skin over the ulcer site appeared healthy. There was no sign of the fungal lesions that had been present on the right foot and both hands.

Discussion and Conclusions

This participant is a relatively recent paraplegic who developed pressure ulcers on both feet from the foot of the bed and the footplate of her electric wheelchair. She has a past history of eczema and dermatitis and long term steroid use prior to her spinal injury which may have affected the integrity of her skin leading to the chronic pressure ulcers on her feet. The ulcers were first reported in July 2008 but it was the ulcer on the left foot which was most severe and which continued to break down and require active intervention. Treatment was applied according to a wound care plan with best practice dressings supported by antibiotic therapy and debridement when required. At times non-compliant with medical advice to remain in bed to allow the ulcer time to heal, the participant was also noted to have spent more than the recommended time in her electric wheelchair placing additional pressure on the affected area. This ulcer had been present for nearly seven months, waxing and waning in size, severity and associated infection prior to initiation of treatment with OPALA filtrate and cream on 20 January 2009. Only one further (six day) course of antibiotics was prescribed on 2 February 2009 for infection in the wound. The ulcer remained clean and clear of slough and infection and was completely healed after eight weeks of treatment with OPALA filtrate and cream. Use of the cream continued for a further three weeks as the skin around the affected area was dry and fragile. At the time of reporting, six weeks after of the ulcer had been reported by nursing and medical staff as having healed, there was no sign of recurrence of ulceration when the participant's feet were examined and the skin over the previous ulcer site appeared healthy. In addition, examination of the hands and feet did not indicate any clinical evidence of fungal infection.